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RELATIONSHIPS IN THE GENUS GOSSYPIUM AS INDICATED BY CYTOLOGICAL DATA[1]

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INTRODUCTION

In recent years several taxonomic, genetic, and cytologic studies of *Gossypium* have been conducted with the purpose of determining the phylogenetic relationships within the genus. As a result of these studies four groups are now recognized. The present report surveys the cytogenetic data bearing upon the relationships existing within and among these four groups of *Gossypium*.

SUBDIVISIONS OF THE GENUS GOSSYPIUM

The four groups now recognized in the genus *Gossypium* are as follows:

(1) A wild Australian species, *Gossypium sturtii* F. Muell. This is one of the most distinct species of the genus. Although it is generally separated from other groups of *Gossypium*, it was included in the Asiatic group in Skovsted's recent cytological classification (*29*).[2]

(2) Asiatic species. This group includes wild and cultivated species from both Asia and Africa. Although there have been 12 or more cultivated Asiatic species described, Watt (*31*) recognizes only four, namely, *Gossypium arboreum* L., *G. nanking* Meyen, *G. obtusifolium* Roxb., and *G. herbaceum* L. Recently Harland (*17*) and Hutchinson (*19*) reduced the cultivated Asiatic cottons to two species, *G. arboreum* and *G. herbaceum*. They included in this group two wild species, *G. anomalum* Wawra and Peyr. from French Sudan and Somaliland, and *G. stocksii* M. Mast. from Sind and southeastern Arabia.

(3) Wild American species. This group includes the following species with 13 pairs of chromosomes of southwestern North America and the Galápagos Islands: *Gossypium armourianum* Kearney, *G. harknessii* Brandeg., *G. thurberi* Tod., *G. davidsonii* Kellogg, *G. klotzschianum* Anderss., and possibly *G. raimondii* Ulbr. of Peru and *G. trilobum* (DC.) Kearney (*20*), *G. gossypioides* (Ulbr.) Standl., and *Erioxylum aridum* Rose and Standl. (*G. aridum* Skovsted) of Mexico (*29*).

(4) Cultivated American species. All of the species with 26 pairs of chromosomes, whether from North America, South America, Africa, or the islands of the Pacific Ocean, are included in the cultivated American group. Although 20 or more species have been described, Harland (*17*) recognizes only the following 5: (1) Upland type, *Gossypium hirsutum* L.; (2) Bourbon type, *G. purpurascens* Poir; (3) Peruvian type, *G. barbadense* L.; and the Polynesian wild cottons;

[1] Received for publication August 19, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 259.

(4) *G. tomentosum* Nutt. (Hawaiian Islands) and (5) *G. taitense* Parl. (Fiji Islands, etc.).

EARLY HISTORY OF THE CYTOGENETICS OF GOSSYPIUM

Interspecific hybrids in *Gossypium* were made as early as 1790 by Rohr (Watt (*31*)), who recognized the possibility of improving the cotton crop by means of hybridization. In 1872 Clark (Watt (*31*)), reported that it was fairly easy to cross species of cotton within the Asiatic group or within the cultivated American group but that it was impossible to produce hybrids between the groups. Until 1923 Clark's report was generally accepted as correct, since all attempts to produce a hybrid between an Asiatic and an American species had resulted in failure. On the other hand, F_1 hybrids within the Asiatic and the cultivated American group, respectively, were found to be easily made and highly fertile and consequently capable of utilization in the improvement of cotton.

In 1923 Zaitzev (*39*) found the first recorded hybrid between a cultivated Asiatic and a cultivated American cotton and reported that it was completely sterile. In 1927 he (*40*) reported an artificially made Asiatic-American hybrid and described five natural hybrids between members of these groups. In the same year Desai (*11*) obtained one hybrid from numerous crosses between species of the two groups. Recently approximately 20 artificial and natural Asiatic-cultivated American hybrids have been reported by Zhurbin according to Emme (*12*), Nakatomi (*22*), Harland (*16*), Szymanek and Gavaudan (*30*), Baranov (*4*), Longley (*21*), Feng (*13*), and Skovsted (*25*).

Cytological investigations of *Gossypium* began in 1903 with Cannon's studies of the cultivated American hybrid *G. barbadense* × *G. hirsutum*. He (*6*) reported that the hybrid had 28 haploid chromosomes and that it exhibited complete chromosome conjugation and normal disjunction during the heterotypic division. Later Ball (*2*) reported *G. barbadense* to possess 20 haploid chromosomes. In 1923 and 1924 Nikolajeva (Zaitzev (*39*)) and Denham (*9*, *10*) established 13 as the haploid chromosome number for Asiatic species of *Gossypium* and 26 as the haploid chromosome number for the cultivated American species. At the same time Denham (*10*) reported complete chromosome conjugation in two Asiatic hybrids, *G. cernuum* × *G. rudicum*[3] and *G. arboreum* × *G. neglectum*. That all cultivated or wild Asiatic species have 13 pairs and all cultivated American species have 26 pairs of chromosomes has been confirmed by Youngman and Pande (*38*), Longley (*21*), Beal (*5*), Banerji (*3*), and Webber (*33*). Harland (*15*), Longley (*21*), and Webber (*33*) have found that *G. sturtii* and all wild American species of *Gossypium* have 13 haploid chromosomes.

In 1930 Baranov (*4*) reported that a cultivated American-Asiatic hybrid (*Gossypium hirsutum* × *G. herbaceum*) formed 13 bivalent and 13 univalent chromosomes during meiosis. He also observed that the univalents were distributed irregularly. Similar observations were reported in other cultivated American-Asiatic hybrids by Nakatomi (*22*) in 1931, and Zhurbin as reported by Emme (*12*) in 1930. Longley (*21*) reported variable chromosome conjugation in a natural hybrid of this type.

[3] Name as given in Denham's paper, probably a misprint for *G. indicum* Lam. The latter is supposed to be the same as *G. nanking* Meyen.

SUMMARY OF RECENT CYTOGENETIC STUDIES OF GOSSYPIUM

Since 1934 there have been produced intergroup hybrids between species representing each of the four groups of *Gossypium* and between most of the distinctly different species within each of the groups. Most of these hybrids have been studied cytologically. The important interspecific hybrids and a few variant plants that have bearing on the phylogeny of the genus are listed in table 1, together with their reported meiotic chromosome pairing and fertility. The names of species as given in the table, are, for the most part, those under which the original seeds were received. It is very doubtful that many of the "species" that have been described in the cultivated Asiatic group and in the cultivated (and semiwild) American group are really entitled to this rank.

CHROMOSOME MORPHOLOGY

Skovsted (*25*) divided the species of *Gossypium* with 13 pairs of chromosomes into a group possessing large somatic chromosomes and a group having small somatic chromosomes. He placed *G. sturtii* in the group characterized by larger somatic chromosomes. Later he (*26*) depicted both the somatic and meiotic chromosomes of *G. sturtii* and reported that its meiotic chromosomes also were larger than those of species belonging to the group with smaller somatic chromosomes. According to the observations of the writer, the somatic chromosomes of *G. sturtii* are larger than those of the majority of species of *Gossypium* with 13 pairs of chromosomes, but the meiotic chromosomes are not correspondingly large.

According to Baranov (*4*), the somatic chromosomes of the cultivated Asiatic species exhibit such morphological distinctions as differences in length of arms, divided and undivided arms, arms with or without satellites, etc. Later Skovsted (*23*, *25*) reported that the Asiatic species are characterized by 13 pairs of relatively large chromosomes and that the 26 somatic chromosomes of *Gossypium arboreum* are all of equal size and 4 of them possess satellites. After a careful study of *G. herbaceum*, Arutjunova (*1*) divided the somatic complement into 13 types, noting that several of the chromosome types resembled one another and that 4 of them were much smaller than the others. Both Denham (*10*) and Davie (*8*) observed that one pair of chromosomes in the Asiatic cottons is longer than the others, and Davie found that the species of this group exhibited considerable secondary pairing. The writer agrees that the Asiatic species form a group characterized by 13 pairs of relatively large chromosomes but has found that the somatic chromosomes of Asiatic species are slightly smaller than those of *G. sturtii*. Neither Skovsted nor Arutjunova found the larger pair of chromosomes reported by Denham and Davie, and neither Webber (*33*) nor Skovsted has found secondary association in any Asiatic species.

Skovsted (*25*) classified the wild American species of *Gossypium* as a group having 13 pairs of smaller chromosomes. He (*26*) figured the somatic chromosomes of *G. davidsonii* as approximately one-half the length and one-half the diameter of those of *G. sturtii*, and later (*29*) found that secondary pairing in the wild American species is more noticeable than in other species with 13 pairs of chromosomes.

TABLE 1.—*Summary of the meiotic chromosome conjugation and fertility of interspecific hybrids and variants in Gossypium*

Category of hybrids, whether within a group or between groups, and haploid chromosome numbers of the parent species	Hybrid or variant	Meiotic chromosome behavior [1]	Fertility [2]	Investigator and reference
CATEGORY 1 Asiatic ($n=13$) × Asiatic ($n=13$). (Group 2 × group 2).	*G. arboreum* × *G. neglectum*	13_{II}		Denham (*10*).
	G. cernuum × *G. rudicum* (sic)	13_{II}		Do.
	G. sanguineum × *G. africanum*	13_{II}; occasionally 2_{I}	Highly fertile	Webber (*35*).
	G. herbaceum × *G. neglectum*	13_{II}	do	Do.
	G. herbaceum × *G. cernuum*	Mean: $0.20_{I}+12.9_{II}$	do	Webber (present article).
	G. herbaceum × *G. arboreum*	13_{II}; occasionally a few univalent	Fertile	Skovsted (*28*).
	G. herbaceum × *G. arboreum*, triploid	Mean: $2.5_{I}+5.7_{II}+6.0_{III}+1.1_{IV}+3.5_{V}+0.1_{VI}+0.05_{VII}$	Sterile	Do.
	G. sanguineum × *G. anomalum*	Mean: $2.36_{I}+11.82_{II}$	40 percent fertile	Webber ((*36*) and present article).
	G. arboreum × *G. anomalum*	Mean: $3.1_{I}+10.45_{II}+0.2_{III}+0.35_{IV}$		Skovsted (*29*).
	G. nanking × *G. anomalum*, 2 hybrids	Mean: $1.65_{I}+11.70_{II}+0.15_{III}+0.125_{IV}$		Do.
	G. africanum × *G. anomalum*, 2 hybrids	Mean: $2.5_{I}+11.75_{II}$		Do.
	(*G. herbaceum* × *G. neglectum*) × *G. stocksii*.	Mean: $24.60_{I}+0.70_{II}$	Sterile	Webber ((*36*) and present article).
	G. sanguineum × *G. stocksii*	Mean: $24.10_{I}+0.95_{II}$	Slightly fertile	Do.
	G. arboreum × *G. stocksii*, 5 hybrids	Mean: $13.48_{I}+6.25_{II}$		Skovsted (*29*).
	G. nanking × *G. stocksii*, 5 hybrids	Mean: $17.3_{I}+4.35_{II}$		Do.
	G. wrightiana × *G. stocksii*, 5 hybrids	Mean: $19.6_{I}+3.2_{II}$		Do.
	G. harknessii × *G. armourianum*	13_{II}	Highly fertile	Webber (*35*).
	G. armourianum × *G. thurberi*, 2 hybrids.	Mean: $0.60_{I}+12.70_{II}$	85 percent fertile	Webber ((*37*) and present article)
CATEGORY 2 Wild American ($n=13$) × wild American ($n=13$). (Group 3 × group 3).	*G. thurberi* × *Eriozylum aridum* (*G. aridum*).	13_{II}	Sterile	Skovsted (*27*, *29*).
	G. armourianum × *G. thurberi*	13_{II}; 1 pollen mother cell out of 45= $2_{I}+12_{II}$.	Fertile	Skovsted (*29*).
	G. armourianum × *Eriozylum aridum* (*G. aridum*).	13_{II}		Do.
	G. davidsonii × *G. klotzschianum*	Mean: $0.10_{I}+12.95_{II}$	90 percent fertile	Webber (present article).
	G. harknessii × *G. thurberi*	Mean: $0.80+12.60_{II}$	70 percent fertile	Do.

	G. hirsutum × *G. punctatum*	26_{II}; occasionally 1, 2, or 3_{IV}	Highly fertile	Webber (*35*).
	G. hirsutum × *G. barbadense*	do	do	Do.
	G. contextum × *G. schottii*	do	do	Do.
	G. barbadense × *G. schottii*	do	do	Do.
	G. barbadense × *G. punctatum*	do	do	Do.
	G. barbadense × *G. tomentosum*	26_{II} in 5 pollen mother cells		Skovsted (*29*).
CATEGORY 3	*G. hirsutum* × *G. barbadense*	26_{II} in 5 pollen mother cells; 2_I+25_{II} in 1 pollen mother cell.		Do.
Cultivated American ($n=26$) × cultivated American ($n=26$). (Group 4 × group 4).	*G. peruvianum* × *G. darwinii*	Mean: $25.7_I+0.15_{IV}$	Highly fertile	Webber (present article).
	G. contextum × *G. hopi*	Mean: $0.20_I+25.7_{II}+0.10_{IV}$	do	Do.
	G. barbadense × *G. tomentosum*	26_{II}	do	Do.
	G. hirsutum × *G. tomentosum*, 2 hybrids.	26_{II}	do	Do.
	G. purpurascens × *G. tomentosum*	26_{II}	do	Do.
	G. sp., haploid	Few trisomes		Skovsted (*23*).
	G. barbadense, haploid	Mean: $25.6_I+0.20_{II}$	Slightly fertile	Webber (*37*).
	G. sturtii × *G. armourianum*	Mean: $24.08_I+0.96_{II}$	Sterile	Webber (*35*).
CATEGORY 4	*G. sturtii* × *G. harknessii*	Mean: $24.56_I+0.72_{II}$	Slightly fertile	Do.
	G. davidsonii × *G. sturtii*	Mean: $23.94_I+1.03_{II}$	Sterile	Do.
G. sturtii ($n=13$) × wild American ($n=13$). (Group 1 × group 3).	*G. thurberi* × *G. sturtii*	Mean: $24.52_I+0.74_{II}$	do	Webber ((*35*) and present article).
	G. davidsonii × *G. sturtii*	Mean: $14.95_I+5.33_{II}+0.1_{III}+0.03_{IV}$ 90 percent allosyndetic conjugation.	do	Skovsted (*26*).
	G. sturtii × *G. armourianum*	Mean: $8.45_I+8.20_{II}+0.25_{III}+0.1_{IV}$		Skovsted (*29*).
	G. nanking × *G. thurberi*	Mean: $23.95_I+1.025_{II}$	Very slightly fertile	Webber ((*36*) and present article).
	G. arboreum × *G. thurberi*, 3 hybrids	Mean: $10.08_I+7.58_{II}+0.2_{III}+0.04_{IV}$		Skovsted (*29*).
	G. nanking × *G. thurberi*, 2 hybrids	Mean: $9.25_I+79.25_{II}+0.2_{III}+0.075_{IV}$		Do.
CATEGORY 5	*G. anomalum* × *Eriozylum aridum* (*G. aridum*).	Mean: $21.15_I+2.35_{II}+0.05_{III}$	Sterile	Skovsted (*27*, *29*).
Asiatic ($n=13$) × wild American ($n=13$). (Group 2 × group 3).	*G. anomalum* × *G. thurberi*	Mean: $14.3_I+5.7_{II}+0.10_{III}$		Skovsted (*29*).
	G. anomalum × *G. davidsonii*	Mean: $19.9_I+3.05_{II}$		Do.
	G. davidsonii × *G. anomalum*	Mean: $25.6_I+0.2_{II}$	Sterile	Webber (present article).
CATEGORY 6	*G. herbaceum* × *G. sturtii*	Mean: $21.56_I+2.22_{II}$	do	Webber ((*36*) and present article).
Asiatic ($n=13$) × *G. sturtii* ($n=13$). (Group 2 × group 1).	*G. arboreum* × *G. sturtii*, 2 hybrids	Mean: $2.85_I+9.85_{II}+0.65_{III}+0.375_{IV}$	do	Skovsted (*27*, *29*).

See footnotes at end of table.

TABLE 1.—*Summary of the meiotic chromosome conjugation and fertility of interspecific hybrids and variants in Gossypium*—Continued

Category of hybrids, whether within a group or between groups, and haploid chromosome numbers of the parent species	Hybrid or variant	Meiotic chromosome behavior [1]	Fertility [2]	Investigator and reference
	G. sp. × *Eriozylum aridum* (*G. aridum*)	$13_{II}+13_{I}$	Slightly fertile	Skovsted (*24*).
	G. sp. × *G. harknessii*	$13_{II}+13_{I}$	do	Do.
	G. hirsutum × *G. armourianum*	$13_{II}+13_{I}$	do	Webber (*34, 35*).
	G. contextum × *G. armourianum*	Mean: $13_{I}+12.72_{II}+0.14_{IV}$	do	Do.
	G. barbadense × *G. harknessii*	$13_{II}+13_{I}$	do	Do.
	G. hirsutum × *G. thurberi*, 2 hybrids	Mean: $13.06_{I}+12.70_{II}+0.18_{III}$	do	Webber (*36*) and present article).
	G. barbadense × *Eriozylum aridum* (*G. aridum*).	Mean: $13.99_{I}+12.28_{II}+0.15_{III}$	Somewhat pollen-fertile	Skovsted (*29*).
CATEGORY 7	*G. hirsutum* × *Eriozylum aridum* (*G. aridum*).	Mean: $13.15_{I}+12.4_{II}+0.35_{III}$		Do.
Cultivated American ($n=26$) × wild American ($n=13$). (Group 4 × group 3).	*G. purpurascens* × *Eriozylum aridum* (*G. aridum*).	Mean: $13.00_{I}+12.0_{II}+0.6_{III}+0.05_{IV}$		Do.
	G. barbadense × *G. armourianum*	Mean: $14.75_{I}+12.05_{II}+0.05_{III}$	Somewhat fertile	Do.
	G. hirsutum × *G. armourianum*, 2 hybrids.	Mean: $12.67_{I}+12.75_{II}+0.275_{III}$		Do.
	G. purpurascens × *G. armourianum*, 2 hybrids.	Mean: $13.775_{I}+12.35_{II}+0.175_{III}$		Do.
	G. laitense × *G. armourianum*	Mean $14.5_{I}+12.1_{II}+0.1_{III}$		Do.
	G. darwinii × *G. armourianum*	Mean: $13.2_{I}+12.00_{II}+0.6_{III}$		Do.
	G. barbadense × *G. thurberi*	Mean: $12.7_{I}+12.4_{II}+0.5_{III}$	Somewhat fertile	Skovsted (*27, 29*).
	G. hirsutum × *G. thurberi*	Mean: $13.5_{I}+12.45_{II}+0.2_{III}$	do	Do.
	G. laitense × *G. thurberi*	Mean: $13.4_{I}+12.05_{II}+0.5_{III}$		Skovsted (*29*).
	G. hirsutum × *G. herbaceum*	$13_{I}+13_{II}$		Baranov (*4*).
	G. hirsutum × *G. herbaceum*	$13_{I}+13_{II}$; nondisjunction of few bivalents.	Sterile	Nakatomi (*22*).
	G. barbadense × *G. herbaceum*	do	do	Do.
	G. hirsutum × *G. herbaceum*	$13_{I}+13_{II}$		Zhurbin (Emme (*12*)).
	Natural F_1	Variable conjugation, as high as $11_{I}+14_{II}$.		Longley (*21*).
	G. barbadense × *G. arboreum*	Mean: $14.43_{I}+8.43_{II}+1.03_{III}+0.63_{IV}+0.25_{V}+0.15_{VI}$.	Slightly fertile	Skovsted (*25*).
CATEGORY 8	*G. nanking* × *G. hirsutum*	$13_{I}+13_{II}$, nondisjunction of few bivalents.	Self-sterile	Feng (*13*).
Cultivated American ($n=26$) × Asiatic ($n=13$). (Group 4 × group 2).	*G. hirsutum* × *G. nanking*		Somewhat cross-fertile	
	Natural F_1	Mean: $13.71_{I}+11.94_{II}+0.35_{IV}$		Webber (*35*).
	G. hirsutum × *G. cernuum*	Mean: $13.22_{I}+12.74_{II}+0.1_{III}$ rarely nondisjunction of 1 or 2_{II}.	Somewhat fertile	Webber (present article).
	(*G. barbadense* × *G. schottii*) × *G. anomalum*.	Mean: $18.12_{I}+10.32_{II}+0.08_{III}$	Sterile	Webber (*36*) and present article).
	(*G. contextum* × *G. kopi*) × *G. anomalum*.	Mean: $17.81_{I}+10.48_{II}+0.11_{III}$	do	Do.
	G. barbadense × *G. anomalum*	Mean: $33.80_{I}+2.6_{II}$		Skovsted (*29*).
	G. barbadense × *G. stocksii*	Mean: $37.9_{I}+0.55_{II}$		Do.
	(*G. arboreum* × *G. herbaceum*) × (*G. hirsutum* × *G. barbadense*) $2n=52$.	Mean: $15.75_{I}+5.90_{II}+5.95_{III}+1.0_{IV}+0.4_{V}+0.1_{VI}$.	Sterile	Skovsted (*25*).

CATEGORY 9	*G. barbadense* × *G. sturtii*	Mean: $37.18_{I}+0.91_{II}$	do	Webber (*34*, *35*).
	G. barbadense × *G. sturtii*	Mean: $32.5_{I}+3.10_{II}+0.1_{III}$	do	Skovsted (*29*).
Cultivated American ($n = 26$) × *G. sturtii* ($n = 13$). (Group 4 × group 1).	*G. hirsutum* × *G. sturtii*	Mean: $20.95_{I}+7.6_{II}+0.95_{III}$	do	Do.
	G. purpurascens × *G. sturtii*	Mean: $24.33_{I}+6.0_{II}+0.33_{III}+0.05_{IV}$	do	Do.
	G. darwinii × *G. sturtii*	Mean: $27.1_{I}+5.25_{II}+0.4_{III}+0.05_{IV}$	do	Do.
	G. hirsutum × *G. sturtii*, 3 hybrids	Mean: $37.92_{I}+0.52_{II}$	do	Webber (present article).

[1] The subscripts I, II, III, etc., are cytological symbols designating univalent, bivalent, trivalent, quadrivalent, pentavalent, hexavalent, and septavalent chromosomes.

[2] The percentage of fertility, indicated in the table and in the following paragraphs, was determined from the number of apparently good seeds in 3 or more selfed bolls and the number of apparently poor seeds and undeveloped ovules in the same bolls.

The writer has not observed secondary pairing in the wild American species.

Skovsted (*25*) reported that in cultivated American cottons 13 chromosomes are larger than the other 13; the larger ones being comparable in size to the chromosomes of Asiatic cottons and the smaller ones being equal in size to those of the wild American species. On the other hand, Arutjunova (*1*) found that while both *Gossypium hirsutum* and *G. herbaceum* have large and small chromosomes the chromosomes of *G. hirsutum* duplicate the small and large types of chromosomes in the Asiatic species. According to this investigator the chromosomes of *G. hirsutum* are in general somewhat smaller than those of Asiatic species. Webber (*35*) reported that the differences in chromosome size in cultivated American species are very slight and that there is a gradation of sizes rather than a sharp distinction between large and small chromosomes. Beal (*5*) found chromosome size to be of uniform gradation from very small to relatively large. Denham (*10*) and Davie (*8*) observed that two pairs of chromosomes in the cultivated American cottons were longer than the others. Secondarily paired bivalent chromosomes and quadrivalent chromosomes have been reported (*35*) in the cultivated American species. In haploid cultivated American species (table 1, category 3), Skovsted reported a few trisomes and Webber noted the occurrence of bivalent chromosomes.

INTERSPECIFIC HYBRIDS

HYBRIDS INVOLVING GOSSYPIUM STURTII (GROUP 1)

Cross-pollinations between *Gossypium sturtii* and species belonging to other groups of *Gossypium* are easily obtained. The F_1 plants are the most nearly sterile of any of the intergroup hybrids. In the past 3 years approximately 110 hybrids involving *G. sturtii* as one of the parents were grown. Although these plants flowered profusely during this period, they produced only two open-pollinated seeds. These seeds, of *G. sturtii* × *G. harknessii*, produced F_2 plants resembling the intermediate F_1.

The meiotic chromosome pairing in hybrids between cultivated American species and *G. sturtii* is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of three F_1 hybrids (W 81, W 82, and W 139) between *G. hirsutum* and *G. sturtii*.

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
37_I+1_{II}	8
35_I+2_{II}	5
33_I+3_{II}	3
39_I	34

Mean conjugation = $37.92_I+0.52_{II}$.

The foregoing tabulation shows that during meiosis there is a mean conjugation of 37.92 univalents and 0.52 bivalents in *Gossypium hirsutum* × *G. sturtii*. Although Skovsted (*29*) reported considerably more variable conjugation and the occurrence of a few trivalents and quadrivalents in similar hybrids, the mean conjugation in all ob-

served hybrids between cultivated American species and *G. sturtii* (table 1, category 9) is similar.

The meiotic chromosome conjugation in Asiatic species × *G. sturtii* is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of *G. herbaceum* × *G. sturtii* F_1 (W 111).

Types of conjugation in 40 pollen mother cells:	*Number of pollen mother cells*
26_I	7
24_I+1_{II}	8
22_I+2_{II}	10
20_I+3_{II}	7
18_I+4_{II}	4
16_I+5_{II}	2
14_I+6_{II}	1
12_I+7_{II}	0
10_I+8_{II}	1

Mean conjugation = $21.56_I+2.22_{II}$.

In the foregoing tabulation it is shown that *Gossypium herbaceum* × *G. sturtii* F_1 forms 10 to 26 univalent and 0 to 8 bivalent chromosomes, with a mean conjugation of $21.56_I+2.22_{II}$ during meiosis. In similar hybrids Skovsted (*29*) observed a range of 0 to 14 univalents and 6 to 13 bivalents, with a mean of $2.85_I+9.85_{II}+0.65_{III}+0.375_{IV}$.

The writer (*35*) reported that there was no visible chromosome conjugation in *Gossypium thurberi* × *G. sturtii*. A further study of this hybrid, involving greater numbers of pollen mother cells, showed that it exhibited a limited amount of variable pairing. Following are the data on the first metaphase chromosome conjugation in pollen mother cells of *G. thurberi* × *G. sturtii* F_1 (W 58).

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
26_I	29
24_I+1_{II}	11
22_I+2_{II}	6
20_I+3_{II}	2
18_I+4_{II}	2

Mean conjugation = $24.52_I+0.74_{II}$.

In analogous hybrids Webber found (table 1, category 4) the mean numbers of univalent chromosomes to range from 23.94 to 24.56 and those of bivalent chromosomes from 0.72 to 1.03. Skovsted (*26*, *29*) reported that the mean conjugation in similar hybrids was 8.45 to 14.95_I; 5.33 to 8.20_{II}; 0.10 to 0.25_{III}; and 0.03 to 0.10_{IV}. In a detailed study of *Gossypium davidsonii* × *G. sturtii*, Skovsted (*26*) was able to distinguish the parental chromosomes. He stated that the number of univalents is the same from both parents and that about 90 percent of the bivalents are formed by allosyndesis. The autosyndetic bivalents are formed in equal numbers by *G. davidsonii* and *G. sturtii*. Skovsted reported that a maximum of 10 allosyndetic and 2 autosyndetic bivalents were formed in this hybrid.

HYBRIDS INVOLVING ASIATIC SPECIES (GROUP 2)

Within the Asiatic group, excepting the two wild species (*Gossypium anomalum* and *G. stocksii*) interspecific hybrids are easily obtained and are highly fertile. The writer found that *G. anomalum* hybridizes fairly easily with *G. sanguineum* and produces a hybrid which is 40-percent fertile when selfed or backcrossed. *G. stocksii* is rather easily crossed with *G. sanguineum* and with *G. neglectum*. Six F_1 plants of *G. sanguineum* × *G. stocksii* produced only three open-pollinated seeds, and two F_1 plants of *G. neglectum* × *G. stocksii* failed to produce any seeds. Two hybrids between Asiatic species and wild American species, *G. davidsonii* × *G. anomalum* and *G. nanking* × *G. thurberi*, were obtained. The former hybrid is easily produced. Eight F_1 plants were grown but failed to produce a single seed. *G. nanking* × *G. thurberi* is one of the most difficult *Gossypium* hybrids to make. From nine F_1 plants [4] four open-pollinated seeds were collected. The writer obtained three hybrids between Asiatic species and cultivated American species. Although several natural hybrids between these groups have been reported, such hybrids are, nevertheless, among the most difficult to produce. A single F_1 plant of *G. hirsutum* × *G. cernuum* produced four open-pollinated seeds. On the other hand, one F_1 plant of the compound hybrid *G.* (*barbadense* × *schottii*) × *G. anomalum* and three F_1 plants of *G.* (*contextum* × *hopi*) × *G. anomalum* failed to form any seeds. Hybrids between Asiatic species and *G. sturtii* have been discussed in the preceding section.

The majority of plants in segregating generations of *Gossypium sanguineum* × *G. africanum* and *G. herbaceum* × *G. neglectum* differ considerably from each other and are highly fertile. Several F_3 plants of *G. sanguineum* × *G. africanum* resembled a different or third species, and one in particular resembled *G. nanking*. Five out of eight F_2 plants of *G. sanguineum* × *G. anomalum* failed to produce any seeds. The remaining three plants were, respectively, 5-, 12-, and 45-percent fertile. Phenotypically two of the plants resembled the *G. anomalum* parent, two the *G. sanguineum* parent, three the F_1 hybrid (intermediate), and one was similar to *G. africanum*. During germination experiments the three seeds from open-pollination of *G. sanguineum* × *G. stocksii* F_1 proved to be only empty seed coats. One of the four seeds from *G. nanking* × *G. thurberi* F_1 germinated. Since it gave rise to conjoined diploid twins, the formation of the seed probably involved sporophytic budding rather than fertilization (*37*). Three plants of *G. hirsutum* × *G. cernuum* F_2 were grown from seeds resulting from open pollination. They strongly resembled the *hirsutum* parent and were, respectively, 30-, 52-, and 65-percent fertile.

The meiotic chromosome pairing in hybrids between species within the Asiatic group is shown in the following data on the first metaphase chromosome conjugation in pollen mother cells of *Gossypium herbaceum* × *G. cernuum* F_1 (W 3).

Types of conjugation in 20 pollen mother cells:	*Number of pollen mother cells*
2_I+12_{II}	2
13_{II}	18
Mean conjugation = $0.20_I+12.9_{II}$.	

[4] In cases where several plants have been grown of hybrids that are difficult to produce, the majority of them are cuttings from a single successful cross-pollination.

Following are the data on the meiotic chromosome conjugation in pollen mother cells of *G. sanguineum* × *G. anomalum* F_1 (W 27).

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
13_{II}	26
2_I+12_{II}	8
4_I+11_{II}	5
6_I+10_{II}	6
8_I+9_{II}	3
10_I+8_{II}	1
12_I+7_{II}	1

Mean conjugation = $2.36_I+11.82_{II}$.

The meiotic chromosome pairing between species within the Asiatic group is likewise shown by the data given in table 2.

TABLE 2.—*Meiotic chromosome conjugation in pollen mother cells of the compound hybrid Gossypium (herbaceum × neglectum) × G. stocksii F_1 (W 98) and of G. sanguineum × G. stocksii F_1 (W 99)*

Types of conjugation in 40 pollen mother cells	Pollen mother cells in—	
	G. (herbaceum×neglectum)×G. stocksii	*G. sanguineum×G. stocksii*
	Number	*Number*
26_I	12	11
24_I+1_{II}	4	3
22_I+2_{II}	3	3
20_I+3_{II}	0	2
18_I+4_{II}	1	1
	Chromosomes	*Chromosomes*
Mean conjugation	$24_I+0.70_{II}$	$24.1_I+0.95_{II}$

Although *Gossypium herbaceum* × *G. cernuum* F_1 generally forms 13 bivalent chromosomes during meiosis, occasionally 2 univalents are present. The mean conjugation, 0.20 univalents and 12.9 bivalents, is comparable to that reported in analogous hybrids (table 1, category 1).

The data in table 2 and in the tabulation immediately preceding it show that hybrids within the Asiatic species group that involve either *Gossypium anomalum* or *G. stocksii* exhibit variable chromosome conjugation.

The data on *Gossypium sanguineum* × *G. anomalum* F_1 show that during meiosis this hybrid forms from 7 to 13 bivalent and from 0 to 12 univalent chromosomes, with a mean conjugation of 2.36 univalents and 11.82 bivalents. In analogous hybrids, Skovsted also found considerable pairing (table 1, category 1). Although chromosome conjugation in *G. (herbaceum* × *neglectum)* × *G. stocksii* and *G. sanguineum* × *G. stocksii* is likewise variable, the number of pairs is considerably less. In these hybrids, from 0 to 4 bivalent and from 18 to 26 univalent chromosomes are formed. The mean numbers of bivalents observed by the writer (table 2) were 0.7 and 0.95, while Skovsted reported (table 1, category 1) mean bivalent numbers of 3.2, 4.35, and 6.25 in similar hybrids.

The meiotic chromosome pairing in hybrids between Asiatic species and wild American species is shown in table 3.

TABLE 3.—*Meiotic chromosome conjugation in pollen mother cells of Gossypium davidsonii × G. anomalum F_1 (W 94) and G. nanking × G. thurberi F_1 (W 79)*

Types of conjugation in 80 pollen mother cells	Pollen mother cells in—	
	G. davidsonii × G. anomalum	*G. nanking × G. thurberi*
	Number	*Number*
26_I	34	18
24_I+1_{II}	4	9
22_I+2_{II}	2	8
20_I+3_{II}	0	4
18_I+4_{II}	0	1
	Chromosomes	*Chromosomes*
Mean conjugation	$25.6_I+0.2_{II}$	$23.95_I+1.025_{II}$

Table 3 shows that the number of bivalent chromosomes during the heterotypic division of *Gossypium davidsonii* × *G. anomalum* F_1 and *G. nanking* × *G. thurberi* F_1, respectively, ranges from 0 to 2, with a mean of 0.2, and from 0 to 4, with a mean of 1.025. In similar hybrids (table 1, category 5) Skovsted found considerably more meiotic pairing, with the occasional formation of trivalent and quadrivalent chromosomes.

The meiotic chromosome pairing in hybrids between Asiatic species and cultivated American species is summarized in the following data on the meiotic chromosome conjugation in pollen mother cells of *G. hirsutum* × *G. cernuum* F_1 (W 21) and in table 4.

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
17_I+11_{II}	2
15_I+12_{II}	4
13_I+13_{II}	41
$12_I+12_{II}+1_{III}$	1
$11_I+11_{II}+2_{III}$	2

Mean conjugation = $13.22_I+12.74_{II}+0.1_{III}$.

TABLE 4.—*Meiotic chromosome conjugation in pollen mother cells of the compound hybrids Gossypium (contextum × hopi) × G. anomalum* F_1 (W 55) *and G. (barbadense × schottii) × G. anomalum* F_1 (W 1)

Types of conjugation in 150 pollen mother cells	Pollen mother cells in—	
	G. (contextum × hopi) × G. anomalum	*G. (barbadense × schottii) × G. anomalum*
	Number	*Number*
27_I+6_{II}	3	1
25_I+7_{II}	4	2
23_I+8_{II}	9	5
21_I+9_{II}	10	6
19_I+10_{II}	17	12
17_I+11_{II}	21	9
15_I+12_{II}	18	8
13_I+13_{II}	12	5

Types of conjugation in 150 pollen mother cells	Pollen mother cells in—	
	G. (contextum × hopi) × G. anomalum	*G. (barbadense × schottii) × G. anomalum*
	Number	*Number*
$12_I+12_{II}+1_{III}$	3	1
$11_I+11_{II}+2_{III}$	1	0
$10_I+10_{II}+3_{III}$	2	1
	Chromosomes	*Chromosomes*
Mean conjugation	$17.81_I+10.48_{II}+0.11_{III}$	$18.12_I+10.32_{II}+0.08_{III}$

The data on *Gossypium hirsutum* × *G. cernuum* F_1 show that this hybrid generally forms 13 bivalent and 13 univalent chromosomes during meiosis. Occasionally, however, pairing is slightly less and rarely trivalent chromosomes are formed. The pairing in this hybrid

is comparable to that reported for all analogous hybrids except those observed by Skovsted (table 1, category 8). Although Skovsted agrees regarding the number of univalents, he found that quadrivalent, pentavalent, and hexavalent chromosomes are formed occasionally.

Meiotic chromosome conjugation in the compound hybrids *Gossypium* (*contextum* × *hopi*) × *G. anomalum* F_1 and *G.* (*barbadense* × *schottii*) × *G. anomalum* F_1 is the most variable observed by the writer among interspecific hybrids in *Gossypium*. Table 4 shows that conjugation in these hybrids ranges from 6 to 13 bivalents, from 10 to 27 univalents, and from 0 to 3 trivalents, with mean conjugations of $17.81_{I}+10.48_{II}+0.11_{III}$ and $18.12_{I}+10.32_{II}+0.08_{III}$. In *G. barbadense* × *G. anomalum* F_1 Skovsted reported a mean conjugation of $33.80_{I}+2.6_{II}$. Skovsted (table 1, category 8) found slightly less pairing in *G. barbadense* × *G. stocksii* F_1.

The meiotic chromosome conjugation of hybrids between Asiatic species and *G. sturtii* has been discussed in the preceding section.

All plants examined of *G. sanguineum* × *G. africanum* F_2 and F_3 exhibited meiotic chromosome conjugation similar to that of their F_1 parent (table 1, category 1). Only three plants of *G. sanguineum* × *G. anomalum* F_2 were examined. These plants also resembled their F_1 parent in having variable pairing. Although the F_2 plants of *G. hirsutum* × *G. cernuum* (table 1, category 8) occasionally formed trivalent and quadrivalent chromosomes and rarely pentavalents, they generally had $4_{I}+22_{II}$, $1_{I}+25_{II}$, and $1_{I}+26_{II}$.

HYBRIDS INVOLVING WILD AMERICAN SPECIES (GROUP 3)

Hybrids between *Gossypium armourianum* and *G. harknessii* and between *G. davidsonii* and *G. klotzschianum* are easily made. The F_1's are highly fertile when selfed or backcrossed. *G. thurberi* is rather easily crossed with *G. armourianum* and with *G. harknessii*. *G. armourianum* × *G. thurberi* F_1 is approximately 85-percent self-fertile, and *G. harknessii* × *G. thurberi* F_1 is about 70-percent self-fertile. Both hybrids are highly fertile when pollinated by pollen from either parent. On the other hand, numerous cross-pollinations between *G. davidsonii* or *G. klotzschianum* and *G. armourianum*, *G. harknessii*, or *G. thurberi* produced only empty seed coats. However, recent experiments indicate that *G. davidsonii* may be combined with *G. armourianum* or with *G. harknessii* in triple hybrids involving *G. thurberi*.

Hybrids of *Gossypium armourianum*, *G. harknessii*, or *G. thurberi* with cultivated American species are somewhat difficult to produce. Twenty-two plants of *G. hirsutum* × *G. armourianum* F_1 produced 4 seeds from self-pollination and 15 seeds from open pollination; 15 plants of *G. barbadense* × *G. harknessii* F_1 produced 4 seeds from self-pollination and 30 seeds from open pollination; and 5 plants of *G. hirsutum* × *G. thurberi* F_1 produced 2 seeds from self-pollination and 16 seeds from open pollination. These hybrids are slightly more fertile when backcrossed with their cultivated American parent than when selfed.

Mature plants of hybrids of *Gossypium davidsonii* or *G. klotzschianum* with cultivated American species were not obtained. Numerous attempts to cross *G. davidsonii* or *G. klotzschianum* with *G. hirsutum* or *G. barbadense* failed completely or produced only empty seed coats. Although hybrids between *G. purpurascens* and *G. klotzschia-*

num are rather easily produced, all die in the cotyledon stage of development.

Interspecific hybrids between *Gossypium sturtii* and the wild American species, and between Asiatic species and wild American species have been discussed in preceding sections of this paper.

Only a few plants were grown of *Gossypium armourianum* × *G. harknessii* F_2 and *G. klotzschianum* × *G. davidsonii* F_2. In each case these plants resembled the intermediate F_1 plants and were fairly self-fertile. Of 59 plants of *G. armourianum* × *G. thurberi* F_2, 31 produced flowers. Of these, 19 were sterile, 7 were slightly fertile, 4 were moderately fertile, and 1 was highly fertile. Phenotypically the plants were highly variable, ranging from plants similar to *G. armourianum* to plants resembling *G. thurberi*. *G. armourianum* × *G. thurberi* F_1, when backcrossed to *G. thurberi*, is highly fertile and very closely resembles *G. thurberi*. On the other hand, when backcrossed to *G. armourianum*, it is only moderately fertile, although the F_1 parent selected resembled *G. armourianum* very closely. The F_2 of *G. harknessii* × *G. thurberi* was not grown.

Four plants from self-pollination and three plants from open pollination of *Gossypium hirsutum* × *G. armourianum* F_2 were grown. All except one of the plants resembled the *G. hirsutum* parent. One of the F_2 plants from seed resulting from open pollination closely resembled the intermediate condition characteristic of F_1 plants. Plants resulting from self-pollination were, respectively, about 0-, 55-, 60-, and 85-percent fertile. Those resulting from open pollination were approximately 0-, 35-, and 70-percent fertile. Four plants from self-pollination and four plants from open pollination of *G. barbadense* × *G. harknessii* F_2 were grown. One of the plants from self-pollination resembled *G. barbadense* × *G. hirsutum* F_1, whereas the other three resembled the *G. barbadense* parent. They were, respectively, about 50-, 75-, 75-, and 80-percent fertile. Of the plants resulting from open pollination of the F_2, two resembled *G. barbadense*, one resembled *G. hirsutum*, and one resembled *G. barbadense* × *G. hirsutum* F_1. They were, respectively, about 85-, 0-, 80-, and 50-percent fertile. In the inbred lineage 17 F_3 plants were grown from an F_2 plant which was 50-percent fertile and which resembled phenotypically *G. barbadense* × *G. hirsutum* F_1. The phenotype of the F_3 plants varied considerably. In general, 10 of these plants resembled the *G. barbadense* parent, 2 resembled *G. hirsutum*, and 1 each resembled *G. peruvianum*, *G. darwinii*, *G. barbadense* × *G. darwinii* F_1, *G. barbadense* × *G. brasiliense* F_1, and *G. barbadense* × *G. hirsutum* F_1. The plants showed such floral abnormalities as stigmatoid anthers and petals, antheroid stigmas and petals, imbricate petals, and cleistogamous flowers. Five of the F_3 plants were sterile, 10 ranged from approximately 15- to 80-percent fertile, and 2 did not flower.

Only two F_1 plants of the compound hybrid *Gossypium* (*barbadense* × *harknessii*) × *G. barbadense* were grown. These plants resembled *G. barbadense* and were moderately fertile.

No F_2 plants of *G. hirsutum* × *G. thurberi* were grown.

Table 5 summarizes the meiotic chromosome conjugation of interspecific hybrids within the wild American group of species.

TABLE 5.—*Meiotic chromosome conjugation in pollen mother cells of Gossypium davidsonii × G. klotzschianum F_1 (W 10), G. armourianum × G. thurberi F_1 (W 8 and W 9), and G. harknessii × G. thurberi F_1 (W 96)*

Interspecific hybrid	Pollen mother cells showing indicated type of conjugation. Total, 60 pollen mother cells			Mean conjugation
	4_I+11_{II}	2_I+12_{II}	13_{II}	
	Number	*Number*	*Number*	
G. davidsonii × G. klotzschianum		1	19	$0.10_I+12.95_{II}$.
G. armourianum × G. thurberi	2	2	16	$.60_I+12.70_{II}$.
G. harknessii × G. thurberi	3	2	15	$.80_I+12.60_{II}$.

Table 5 shows that hybrids within the wild American group of species generally exhibit 13 bivalent chromosomes, though occasionally as many as 4 univalent chromosomes are present. Similar chromosome pairing has been reported previously in analogous hybrids among wild American species (table 1, category 2). Such pairing is also comparable to that reported by Skovsted (table 1, category 2) in hybrids of *Gossypium thurberi* or *G. armourianum* with *Erioxylum aridum* (*G. aridum*).

The meiotic chromosome conjugation in pollen mother cells of *Gossypium hirsutum* × *G. thurberi* F_1 (W 37 and W 44) is shown in the following tabulation:

Types of conjugation in 50 pollen mother cells:	*Number of pollen mother cells*
17_I+11_{II}	1
15_I+12_{II}	3
13_I+13_{II}	39
$12_I+12_{II}+1_{III}$	4
$14_I+11_{II}+1_{III}$	1
$11_I+11_{II}+2_{III}$	2

Mean conjugation = $13.06_I+12.70_{II}+0.18_{III}$.

Although *Gossypium hirsutum* × *G. thurberi* F_1 generally forms 13 bivalent and 13 univalent chromosomes, it occasionally forms fewer bivalents and rarely forms trivalents. Similar chromosome pairing in different types of wild American and cultivated American interspecific hybrids has previously been reported (table 1, category 7).

The meiotic chromosome behavior in hybrids between wild American species and *Gossypium sturtii* and between wild American species and Asiatic species has been described earlier in this paper.

Several plants each of *Gossypium armourianum* × *G. harknessii* F_2 and *G. klotzschianum* × *G. davidsonii* F_2 were examined. In each case, their meiotic behavior was similar to that of the F_1. Fertile and sterile plants of *G. armourianum* × *G. thurberi* F_2 also exhibited meiotic chromosome conjugation similar to that of the F_1.

Although F_2 plants from seeds resulting from self-pollination of *Gossypium hirsutum* × *G. armourianum* F_1 occasionally exhibited trivalents, the common types of conjugation in these plants were 4_I+22_{II}, 2_I+24_{II}, 1_I+25_{II}, and 13_I+26_{II},[5] whereas two plants from

[5] This plant, of pentaploid nature, probably arose from the union of 39- and 26-chromosome gametes, the 39-chromosome gamete having arisen from a suspended mitosis and the 26-chromosome gamete from a 26<>13 first anaphase distribution. It is interesting that, with the exception of one plant with 4_I+26_{II} and one plant with 1_I+26_{II}, all F_2 and F_3 plants total 26 chromosome units. It is probable that at least one of the gametes concerned in the formation of a zygote must have 26 chromosomes.

seeds resulting from open-pollination had 12_I+14_{II} and 4_I+26_{II}. F_2 plants from self-pollination of *G. barbadense* × *G. harknessii* exhibited 2_I+24_{II}, 26_{II}(?), 1_I+25_{II}, and 26_{II}. Only two plants of *G. barbadense* × *G. harknessii* F_3 (2_I+24_{II}) were examined. One of these plants was 60-percent fertile, of *G. barbadense* type, and had 26_{II}. The other plant was sterile, resembled *G. barbadense* × *G. darwinii* F_1, and had 1_I+25_{II}.

HYBRIDS INVOLVING CULTIVATED AMERICAN SPECIES (GROUP 4)

Hybrids between cultivated American species and the three groups of 13-chromosome species have been discussed in the preceding pages (table 1, categories 7, 8, 9). Interspecific crosses within the cultivated American group are the most successful of any interspecific *Gossypium* crosses. The F_1 plants are highly fertile.

The meiotic chromosome conjugation of six interspecific hybrids within the cultivated American group of species is summarized in table 6.

TABLE 6.—*Meiotic chromosome conjugation in pollen mother cells of six interspecific hybrids (F_1) within the cultivated American group of Gossypium species*

Interspecific hybrid	Pollen mother cells showing indicated type of conjugation (total, 100 pollen mother cells)				Mean conjugation
	2_I+25_{II}	26_{II}	$24_{II}+1_{IV}$	$22_{II}+2_{IV}$	
	Number	*Number*	*Number*	*Number*	
G. peruvianum×*G. darwinii* (W 78)		18	1	1	$25.7_{II}+0.15_{IV}$.
G. contextum×*G. hopi* (W 26)	2	16	2		$0.20_I+25.7_{II}+0.10_{IV}$.
G. barbadense×*G. tomentosum* (W 28)		20			26_{II}.
G. hirsutum×*G. tomentosum* (W 29 and W 80)		20			26_{II}.
G. purpurascens×*G. tomentosum* (W 95)		20			26_{II}.

Although interspecific hybrids within the cultivated American group generally exhibit normal meiotic chromosome conjugation, they occasionally form several univalent and quadrivalent chromosomes (table 6). Behavior similar to that shown in table 6 has previously been reported in other hybrids between cultivated American species (table 1, category 3).

DISCUSSION

GENETICAL NATURE OF CHROMOSOME COMPLEMENTS

The fact that hybrids between the wild American species and *Gossypium sturtii* exhibit very little chromosome conjugation indicates that there is very little, if any, homology within the haploid chromosome sets of these groups. Hence, the evidence that either of these species is of polyploid origin is insufficient. Likewise, the lack of secondary association in Asiatic species and the limited conjugation in hybrids between cultivated Asiatic species and *G. stocksii* indicate that if homology exists within the haploid complements of Asiatic species it is very limited. On the other hand, the occurrence of highly polyvalent chromosomes in triploid *G. herbaceum* × *G. arboreum* F_1 (table 1, category 1) and of similar somatic chromosome pairs in *G. herbaceum* (*1*) supports the assumption that the cultivated Asiatic species are of polyploid nature.

The occurrence of bivalents in haploid *G. barbadense* and the presence of quadrivalents and secondary bivalent association in diploid cultivated American species indicate some homology within the haploid chromosome complement of the cultivated American cottons. Further proof of this homology is found in the formation of trivalents in hybrids between cultivated American species and the 13-chromosome species.

INTERSPECIFIC HYBRIDS AND PHYLOGENY

Since *Gossypium sturtii* gives viable hybrids with species of all other groups of *Gossypium*, it is evident that it is more or less related to each group; but the high degree of sterility and the very limited chromosome conjugation in hybrids involving *G. sturtii* suggest that these relationships are rather distant.

In the case of *Gossypium sturtii* × Asiatic species, and possibly *G. davidsonii* × *G. sturtii*, the findings of Skovsted (*26*) and Webber (*35*) are so unlike as to suggest very different conceptions of the relationships. In *G. arboreum* × *G. sturtii* Skovsted (*27*, *29*) found a degree of conjugation adequate to justify the inclusion of *G. sturtii* in the Asiatic group. As indicated above, the sterility and slight amount of conjugation in an analogous hybrid observed by Webber fail to denote close relationship between *G. sturtii* and Asiatic species. Furthermore, hybrids involving Asiatic species other than *G. stocksii* exhibit chromosome conjugation quite different from that of hybrids of which *G. sturtii* was a parent. Skovsted's observation of 10, out of a possible 13, allosyndetic bivalents in *G. davidsonii* × *G. sturtii* suggests, in the writer's opinion, a fairly close relationship between these species. For reasons similar to those given in the case of *G. sturtii* × Asiatic species, Webber's findings indicate, on the contrary, that *G. davidsonii* and *G. sturtii* are not closely related.

The high fertility and complete meiotic chromosome conjugation of hybrids among cultivated Asiatic species denote that these species are closely interrelated. The similarity of F_2 and F_3 segregates to species other than the parents of the F_1 suggests that the majority of the numerous so-called species of the Asiatic group arose through hybridization. Such derivation supports recent taxonomic conclusions that many of the cultivated Asiatic cottons described as species are not entitled to this rank.

Since hybrids between *Gossypium anomalum* and the cultivated Asiatic species exhibit a moderately high degree of fertility and of chromosome conjugation, it is apparent that the parental species are somewhat closely related. On the other hand, the sterility and lack of chromosome conjugation in the compound hybrid *G.* (*herbaceum* × *neglectum*) × *G. stocksii* show that *G. stocksii* is distantly related to the cultivated Asiatic species. It therefore follows that *G. anomalum* and *G. stocksii* are not closely related, since the chromosomes of *G. anomalum* are partly homologous with those of the cultivated Asiatic species while the chromosomes of *G. stocksii* are not.

That the cultivated Asiatic species and *Gossypium anomalum* (group 2) are not closely related to the wild American species (group 3) is shown by the lack of fertility and of chromosome pairing in hybrids between these groups. No hybrids between *G. stocksii* and the wild American species have yet been produced. In the following paragraphs it is shown that the chromosomes of the majority of wild

American species are cytologically homologous with 13 chromosomes of the cultivated American species. On the other hand, data in table 1, category 8, indicate that the chromosomes of *G. stocksii* lack such affinity for those of the cultivated American species. Hence, the chromosomes of *G. stocksii* and those of the wild American species are undoubtedly nonhomologous, and *G. stocksii* is apparently very distantly related to the wild American species.

The occurrence of complete conjugation between the 13 haploid chromosomes of the cultivated Asiatic species and 13 chromosomes of the cultivated American species [6] indicates a rather close relationship between these groups of species. Similar reasons indicate that *Gossypium anomalum* is somewhat closely related to the cultivated American species, while the lack of chromosome pairing in cultivated American species × *G. stocksii* denotes that they are distantly related.

Gossypium armourianum and *G. harknessii* are undoubtedly very closely related, since they produce highly fertile hybrids that exhibit normal chromosome conjugation. Likewise, all cytogenetic data indicate a close relationship between *G. davidsonii* and *G. klotzschianum*. On the other hand, the probability that *G. armourianum* and *G. harknessii* are phylogenetically distinct from *G. davidsonii* and *G. klotzschianum* is indicated by the failure to obtain hybrids between *G. armourianum* (or *G. harknessii*) and *G. davidsonii* (or *G. klotzschianum*), and by the fact that *G. armourianum* and *G. harknessii* hybridize readily with the cultivated American species whereas *G. davidsonii* and *G. klotzschianum* do not. The latter fact is noteworthy because morphologically *G. davidsonii* and *G. klotzschianum* show greater resemblance to the cultivated American cottons than do *G. harknessii* and *G. armourianum*.

Webber (*36*) and Skovsted (*29*) have found *Gossypium thurberi* and *Erioxylum aridum* to hybridize rather freely with both *G. armourianum* and *G. harknessii*, producing fairly fertile hybrids, which exhibit normal chromosome conjugation. Skovsted (*29*) found that *G. thurberi* × *E. aridum* exhibited complete chromosome pairing. The foregoing data indicate a close relationship between *G. thurberi*, *E. aridum*,[7] *G. armourianum*, and *G. harknessii*. On the other hand, the fact that combination between *G. davidsonii* and *G. armourianum* has been obtained only in triple hybrids involving *G. thurberi* suggests that *G. thurberi* may be an intermediate species. A closer relationship between *G. armourianum* and *G. harknessii* than between these species and *G. thurberi* or *E. aridum* is also indicated by histological data. Webber (*32*) has pointed out that while the mesophyll of *G. armourianum* is similar to that of *G. harknessii*, the mesophyll of *G. thurberi* and of *E. aridum* resembles that of *G. davidsonii* and *G. klotzschianum*. That the relationship of *E. aridum* may not be as close as that of *G. thurberi* to *G. harknessii* and *G. armourianum* is suggested by Webber's (*32*) report that lysigenous cavities such as occur in the pith of *Erioxylum* are not characteristic of the pith of any of these species of *Gossypium*.

[6] That this pairing is between chromosomes of the Asiatic and the American species, rather than among chromosomes within the haploid set of the American species, is shown by the lack of conjugation in haploid *Gossypium barbadense* and in certain hybrids such as *G. hirsutum* × *G. sturtii*.

[7] On the basis of these results, Skovsted (*28*) proposed the transfer of *Erioxylum aridum* Rose and Standley to the genus *Gossypium*, as *G. aridum* Skovsted.

In preceding sections cytological indications are given of relationship between the wild American species, on the one hand, and *Gossypium sturtii* and certain species of the Asiatic group, on the other. Since, however, no hybrids have been obtained between *G. davidsonii* or *G. klotzschianum* and *G. stocksii*, or between *G. davidsonii* or *G. klotzschianum* and the other wild American species, there are no available cytological data indicative of the relationships between *G. davidsonii* or *G. klotzschianum* and *G. stocksii*. On the other hand, since the chromosomes of *G. davidsonii* and *G. anomalum* are nonhomologous, whereas the chromosomes of *G. anomalum* are partly homologous with 13 of those of the cultivated American species, it would be inferred that the chromosomes of *G. davidsonii* and those of *G. anomalum* are both nonhomologous with the same set of chromosomes in the cultivated American species. In this case, however, the evidence is not conclusive, since it is very indirect, and based on limited homology between the chromosomes of *G. anomalum* and those of the cultivated American species.

As in the case of the Asiatic species, the occurrence of complete conjugation between the 13 haploid chromosomes of *Gossypium armourianum*, *G. harknessii*, or *G. thurberi* and 13 of the 26 chromosomes of the cultivated American species indicates a fairly close relationship between these three wild American species and the cultivated American species. However, since the chromosomes of the wild American species and those of the Asiatic species are nonhomologous, it may be inferred that the 13 chromosomes of the wild American species and the 13 chromosomes of Asiatic species pair with different sets of 13 chromosomes in cultivated American cottons.

The normal chromosome conjugation and high fertility of interspecific hybrids among cultivated American species (table 1, category 3) denote a close relationship among the species of this group.

It is apparent that the majority of segregates from combinations between species having 13 and 26 chromosomes, respectively, revert to forms having 26 bivalents. Whether these forms will maintain their distinctive characters or revert to the characters of the parent having 26 pairs has not been determined. It is, however, possible that some of the numerous cultivated American forms may have been derived from hybrids between parents having 13 and 26 chromosomes, respectively.

PHYLOGENETIC CONCEPTIONS

Within the haploid complements of the 13-chromosome species of *Gossypium*, Skovsted (*23*, *26*, *29*) observed autosyndetic pairing in the Asiatic species and *G. sturtii*, and autosyndetic and secondary pairing in the wild American species. He offered two alternative explanations as to the origin of these species: That they are (1) derived from a triple cross of two different but closely allied seven-chromosome species with the same six-chromosome species or (2) from the doubling of two similar six-chromosome sets, and the triple appearance of one chromosome. Davie (*8*) observed that one pair of chromosomes in the Asiatic cottons is longer than the others and that the species of this group exhibited considerable secondary pairing. This led him to conclude that the species with 13 pairs of chromosomes probably arose by end-to-end fusion of certain chromosomes in an allotetraploid with 28 chromosomes.

The writer has found no conclusive evidence that homology exists within the haploid chromosome sets of any of the 13-chromosome species. The limited pairing that occurs in hybrids between species of the different groups characterized by 13 pairs of chromosomes may be between the two species sets of 13 chromosomes rather than within one or both sets. Although Denham (*10*) and Davie (*8*) found that one pair of chromosomes in the Asiatic species is longer than the others, this observation is contrary to the findings of Skovsted (*25*), Arutjunova (*1*), and Webber (*33*). Davie's report that secondary association occurs in Asiatic species was unconfirmed by the observations of Skovsted (*25*) and Webber (*33*), while Skovsted's report (*29*) that secondary pairing takes place in the wild American species is contrary to the findings of Webber (*33*). There is also disagreement between Webber (*35*) and Skovsted (*26*) as to the extent and type of pairing in *Gossypium davidsonii* × *G. sturtii*. Hence, although it would seem that the 13-chromosome species of the genus *Gossypium* may have originated from another genus having a lower basal chromosome number, the evidence as to how they originated or what was the ancestral form is still too meager and contradictory to form the basis for definite conclusions.

It is highly probable that the cultivated American species are polyploids of species having 13 pairs of chromosomes. However, it is still questionable whether they are autopolyploids or allopolyploids. If they are allopolyploids there is no indication as to how close was the relationship between the 13-chromosome species that contributed to their ancestry. Dr. Edgar Anderson has suggested [8] that the cytology of Malvaceae leads to the supposition that many species may actually be autopolyploids although all species that have been investigated behave as allopolyploids. Darlington (*7*) has explained such behavior of autopolyploids as due to low chiasma frequency. In *Gossypium* it has been shown (*29, 33*) that the chiasma frequency per bivalent is relatively high within a species and in hybrids between species of the same group but is considerably lower in hybrids between species of different groups. Hence, the frequency of chiasmata in the cultivated American cottons suggests that these species are true allopolyploids rather than autopolyploids behaving as allopolyploids.

The limited chromosome conjugation in haploid *Gossypium barbadense* and in hybrids involving the cultivated American species and 13-chromosome species also supports the assumption that the cultivated American species are allopolyploids. On the other hand, the formation of polyvalents in triploid Asiatic cotton led Skovsted (*23*) to state that conjugation in the triploid must be due to the triploid offering possibilities which, although present in the diploid, do not normally become realized there. "The pairing in the diploid shows only a 'phenotypic conjugation', while the triploid exhibits more of the 'genotypic conjugation'" * * *. (*23*, p. 243). That the complete amount of chromosome homology is not cytologically expressed in diploid cottons is also indicated by the occurrence of trisomes in haploid cultivated American species (*23*), and by the occurrence of more polyvalent chromosomes and of higher degrees of polyvalency in intergroup *Gossypium* hybrids than within the species.

[8] Letter to the writer dated January 12, 1937.

Skovsted (*25*) concluded that the cultivated American cottons are allopolyploids whose origin may be ascribed to the doubling of the chromosomes in a hybrid between an Asiatic and a wild American species. This hypothesis was suggested by his observation that half of the chromosomes of the cultivated American species were relatively large like those of the Asiatic species and that half were smaller like those of the wild American species. Later Skovsted (*29*), after calling attention to the fact that his hypothesis had been criticized chiefly because it involved an Asiatic species in the origin of the cultivated American species, supported his hypothesis with the following evidence: (1) The haploid complement of the cultivated American species is composed of two nonhomologous sets of 13 chromosomes, one of which is homologous with the haploid complement of Asiatic species and the other homologous with the haploid complement of the wild American species (*24*, *25*, *33*, *34*); (2) the chromosomes of the wild American species apparently conjugate with the set of smaller chromosomes in cultivated American cottons (*29*); (3) the "*R*" gene transferred from *Gossypium arboreum* to *G. hirsutum* acts as a member of the cultivated American cotton series of anthocyanin allelomorphs (*18*); and (4) cytological homology exists throughout the group of New World cottons despite their wide distribution in the Americas and Polynesia.

The genetical behavior of the cultivated American cottons caused Longley (*21*) to infer that they are true tetraploids. Davie (*8*) considered the cultivated American cottons to be allopolyploids and suggested *Gossypium armourianum*, *G. harknessii*, *G. davidsonii*, or *G. klotzschianum* as possible parents. Arutjunova (*1*) considered the species of *Gossypium* with 26 pairs of chromosomes to be amphidiploids, each derived from two very closely related species having morphologically similar sets of chromosomes. Although Gates (*14*) granted that the cultivated American species may have arisen in post-Columbian time from hybrids between Asiatic and wild American species, he favored the hypothesis that they originated from wild American species and suggested that an unknown wild American species may have been involved. The latter hypothesis is supported, in the writer's view, by the following evidence: The presence of two sets of morphologically similar chromosomes in cultivated American species; the formation of quadrivalent chromosomes in diploid and of bivalents in haploid cultivated American cottons; the relatively close geographical proximity, yet isolation from one another, of several of the species of wild American cottons; and the fact that, aside from the absence of long lint hairs on the seeds, the wild American cottons possess all the important phenotypic characters that are found in the cultivated American cottons. In the latter connection it should be noted that several F_2 plants of *G. armourianum* × *G. thurberi* greatly resemble *G. hirsutum* and that certain F_2 and F_3 plants of *G. barbadense* × *G. harknessii* resemble cultivated American species other than *G. barbadense*.

Skovsted (*29*) called attention to homology between the chromosomes of *Gossypium armourianum*, *G. harknessii*, and *G. thurberi*, and the absence of homology between the chromosomes of these species and those of *G. davidsonii* and *G. klotzschianum*. The failure of *G. davidsonii* and *G. klotzschianum* to hybridize with *G. armourianum*,

G. harknessii, or *G. thurberi*, and the production of nonviable hybrids between *G. davidsonii* or *G. klotzschianum* and the cultivated American species, led Skovsted to conclude that allopolyploids could have originated only in hybrids between a species of the Asiatic group and a wild American species of the *G. armourianum*, *G. harknessii*, and *G. thurberi* subgroup. In this connection it should be noted that wild American × Asiatic hybrids are extremely difficult to make. Furthermore, as Skovsted stated, the failure of *G. davidsonii* or *G. klotzschianum* to hybridize with other wild American species may be due to a simple genetic difference. Skovsted (*29*) also reported a close relationship between *G. sturtii* and the Asiatic species, and observed in *G. davidsonii* × *G. sturtii* as many as 10 allosyndetic bivalents out of a possible 13. The writer believes that pairing to this extent indicates a rather close relatonship between these two species.

Skovsted also called attention to the fact that if it should be proved that the cultivated American species were derived solely from wild American species the homology between the chromosomes of the Asiatic species and 13 chromosomes of the cultivated American cottons remains to be accounted for. This is true, unless the existence of such homology should be disproved. The possibility of the cultivated American species being autopolyploids that generally behave as allopolyploids and exhibit autopolyploid behavior only under rare or specific conditions should not be overlooked. It is also possible that *Gossypium davidsonii* or *G. klotzschianum* or some of the less-known wild American species may be proved to possess chromosomes homologous with those of the Asiatic species.

SUMMARY

The cytogenetic literature that has critical bearing on the relationships of species in the genus *Gossypium* is reviewed and summarized in table form.

The meiotic chromosome conjugation is reported of (1) F_1 of 23 new interspecific hybrids, including 12 different types of hybrids; (2) F_2 of certain 13- × 13-chromosome combinations; and (3) F_2 and F_3 of certain 13- × 26-chromosome combinations.

The conjugation observed, in conjunction with that previously reported, strongly supports the grouping of the species as follows: (1) The Australian species, *Gossypium sturtii;* (2) Asiatic species; (3) wild American species; (4) cultivated American species.

The limited meiotic chromosome conjugation of interspecific hybrids involving *Gossypium sturtii* indicates that there is very little, if any, homology within the haploid complement of *G. sturtii* and that its chromosomes are very slightly, if at all, homologous with chromosomes of any other species, with the possible exception of *G. davidsonii*.

The degree of meiotic chromosome conjugation in hybrids involving Asiatic species indicates: (1) That if homology exists within the haploid complement it is very limited; (2) that the cultivated Asiatic species are very closely related to one another; (3) that the wild African species *Gossypium anomalum* is rather closely related to the cultivated Asiatic species; (4) that the wild Asiatic species *G. stocksii* is not closely related to the cultivated Asiatic species and to *G. anomalum;* (5) that within the Asiatic group the relationship to the cultivated American species is closest in the cultivated Asiatic species and

most distant in *G. stocksii*, *G. anomalum* occupying an intermediate position; and (6) that the Asiatic species are not closely related to the wild American species.

Hybridization experiments, meiotic chromosome behavior in F_1, and histological observations indicate that the wild American group is composed of two, or possibly three subgroups: (1) *Gossypium armourianum* and *G. harknessii;* (2) *G. davidsonii* and *G. klotzschianum;* and (3) *G. thurberi*, which appears to be intermediate between subgroups 1 and 2 but more closely related to subgroup 1. *Erioxylum aridum* probably should be included in the genus *Gossypium* and in subgroup 3 of the wild American group. The degree of meiotic chromosome conjugation in hybrids involving wild American species denotes (1) that there is very little, if any, homology between the chromosomes within their haploid complement, and (2) that subgroups 1 and 3 are somewhat closely related to the cultivated American species. It also appears that the chromosomes of these species and of the Asiatic species are homologous with different sets of 13 chromosomes in the cultivated American species. There are no available data that indicate the relationship of *G. davidsonii* and of *G. klotzschianum* to either *G. stocksii* or the cultivated American species. The segregates from wild American species × cultivated American species apparently revert to 26-chromosome forms, which may, at times, resemble 26-chromosome species other than the corresponding parent of the hybrid.

The meiotic chromosome conjugation in haploids, in diploids, and in hybrids involving the cultivated American species indicates (1) that the chromosomes within the haploid complement of the cultivated American species are partly homologous and (2) that the various cultivated American species are closely related.

Three hypotheses as to the origin of 13-chromosome species are discussed, and evidence for and against each hypothesis is given.

Evidence is given that the 26-chromosome species are polyploids, and the possibility is discussed of there being, besides true allopolyploids, autopolyploids that behave as allopolyploids. Evidence is stated for and against the hypothesis of the purely American origin of American cultivated cottons and the alternative hypothesis that they may have originated from the hybridization of an American with an Asiatic species.

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STUDIES OF THE NATURE OF ROOT KNOT RESISTANCE[1]

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INTRODUCTION

During the course of breeding experiments with beans (*Phaseolus vulgaris* L.) resistant to root knot caused by the nematode *Heterodera marioni* (Cornu) Goodey, two observations were made by the writer[3] which raised the question of the nature of root knot resistance. (1) Alabama No. 1 and Alabama No. 2 beans (*P. vulgaris*), which are highly resistant to root knot both in the seedling and adult stage, were observed to form occasional slight swellings immediately behind the root tip when grown in soil badly infested with nematodes. These swellings were never observed to develop into galls. This observation raised the question as to whether Alabama No. 1 and Alabama No. 2 beans resist the entry of nematodes into their roots with the exception of occasional larvae which induce these slight swellings, or whether larvae enter but fail to induce the formation of giant cells and the resulting galls. (2) In the adult stage the Hopi lima beans (*P. lunatus* L.) were consistently less susceptible to root knot under field conditions than other varieties of the species. In the seedling stage, however, no differences could be found between the degree of root knot infection on the Hopi strains and the most susceptible varieties. This suggested an adult plant resistance either of a physiological nature, possibly due to a substance toxic to the nematodes, or of a morphological nature, possibly associated with the tougher root tissue possessed by the Hopi limas.

In the experiments reported in this paper an attempt has been made to elucidate this problem, which is of fundamental interest both to nematologists and breeders working on problems of root knot resistance. The scope of the investigation has been expanded to include the nature of root knot resistance in general.

DEFINITION OF TERMS AND LITERATURE REVIEW

Steiner [4] distinguishes between host resistance to nematode infestation and host immunity. He considers resistant plants to be those that actually resist the entry of nematode larvae by some mechanical or chemical means. Host immunity on the other hand is considered to be the ability of certain plants to show few signs of suffering in spite of the presence of root knot galls. For the purpose of this investigation the term "root knot resistant" will be substituted for "nematode resistant," which has been used by most workers. Root

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[2] The writer is indebted to Dr. G. Steiner, of the Bureau of Plant Industry, U. S. Department of Agriculture, for the loan of an unpublished manuscript by Dr. E. G. Arzberger on the comparative morphology of roots of resistant and susceptible cowpeas.

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knot resistant will be taken to mean any perceptible ability on the part of a plant to grow in nematode-infested soil without the formation of root knot galls. This entirely disregards the important but distinct problem of host tolerance, or host immunity, as it is called by Steiner. In this sense root knot immunity would be taken to mean complete resistance to root knot.

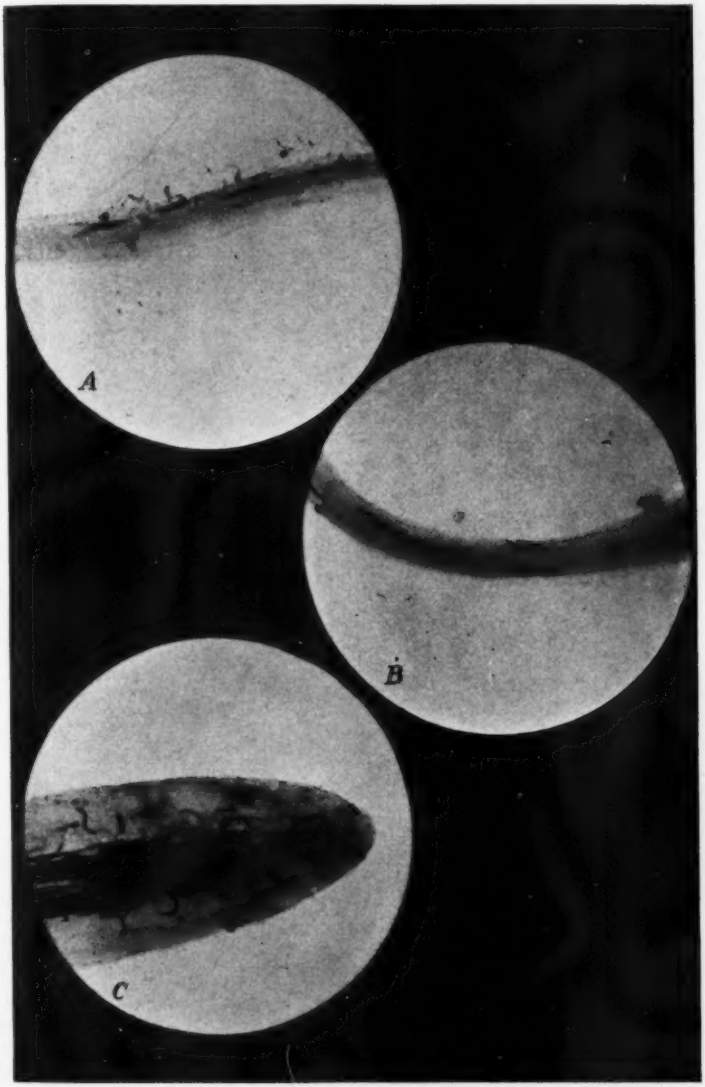
In only one instance, insofar as the writer can determine, has an attempt been made to account for resistance to the root knot disease. In an unpublished investigation Arzberger [5] compared the morphology of the roots of root knot resistant and susceptible cowpeas (*Vigna sinensis* (Torner) Savi). He found that two resistant varieties, Iron and Brabham, had roots that were better guarded by protective tissue than Whippoorwill and Early Buff, which are susceptible. In the resistant varieties the cork layer was also found to be better developed, with fewer broken areas, and the walls of the cork cells were more suberized. The mechanical tissue in the cortex of roots of resistant varieties was found to be more uniformly distributed. Susceptible varieties were found to contain more starch in the cortex, and cells containing starch were more remote from the periphery of the root.

EXPERIMENTAL METHODS AND RESULTS

A microscopical study of bean rootlets was conducted in order to determine whether nematode larvae actually enter root knot resistant varieties and, if so, at what rate as compared with susceptible varieties. Seeds of Alabama No. 1 and Kentucky Wonder beans were planted in 6-inch pots in the greenhouse. The soil was a sandy loam with which had been mixed approximately 20 percent by volume of nematode inoculum prepared by chopping up large root knot galls from adult common bean plants. The seeds were not planted until 3 days after the inoculum was added in order to give the larvae an opportunity to emerge from the root knot tissue and become distributed in the soil. When the secondary roots of the sprouting seeds were between 2 and 4 cm long the seedlings were removed intact by submerging the pots in water and floating them free. Ten vigorous plants each of Alabama No. 1 and Kentucky Wonder were washed free from clinging soil, and 10 rootlets were cut from each plant approximately 1 cm from the tip. The rootlets from each plant were then placed in separate glass vials and treated with hot acetone, followed by Flemming's strong fluid, according to the schedule suggested by Christie.[6] After dehydration, the material was cleared in clove oil and allowed to remain in this medium during the time the studies were being conducted. The larvae were stained black by this procedure, and it was found possible to observe them by the use of dissecting binoculars of the magnification of approximately $\times$ 40. In making routing observations the rootlets were floated in clove oil in a small Petri dish and by manipulation with dissecting needles were passed one at a time through the field of vision. This method permitted a rapid study of the material. Samples of these stained rootlets are shown in plate 1, *A* and *B*.

[5] ARZBERGER, E. G. A COMPARATIVE MORPHOLOGICAL STUDY OF COWPEA ROOTS RESISTANT AND NONRESISTANT TO NEMATODE INFESTATION. Unpublished.

[6] CHRISTIE, J. R. THE DEVELOPMENT OF ROOT-KNOT NEMATODES GALLS. Phytopathology 26: 1-22, illus. 1936.



Root knot larvae in a root of (*A*) Trucker Favorite corn and (*B*) Henderson Bush lima bean. These preparations were stained with Flemming's strong fluid and mounted in balsam. Root tip of Alabama No. 1 bean (*C*) containing so many larvae that it was impossible to count them. The number in a 1-cm section of root was conservatively estimated at 90. Approximately ×44.

The number of larvae observed in each root of the 10 Alabama No. 1 and 10 Kentucky Wonder plants is given in table 1, together with the means for each plant and also the means for the two varieties. It will be seen from the table that considerable variation occurred in the number of larvae found in the rootlets from the same plant. The explanation of the relatively large number found in some rootlets is probably that these rootlets grew near or into a piece of inoculum harboring a large number of larvae. Other rootlets escaped infection altogether. It was not uncommon to observe a number of larvae at one point but nowhere else in the same rootlet.

The generalized standard error calculated on the basis of a 20-plant experiment with 10 replications is 37.66 percent. It will be noted that the mean number of larvae observed in the rootlets of Alabama No. 1 is only slightly lower than that observed in rootlets of Kentucky Wonder. Using the Fisher [7] *t* test for significance of differences, it is readily seen that this difference is not significant at the 5-percent level. Furthermore, using the same test, it is found that the means of no two plants studied differ significantly at the 5-percent level.

TABLE 1.—*Mean number of nematode larvae observed in 10 rootlets each of 10 plants of Alabama No. 1 and Kentucky Wonder beans*

Variety and plant No.	Larvae in root No.—										Mean number per rootlet
	1	2	3	4	5	6	7	8	9	10	
Alabama No. 1:											
1	0	2	2	7	15	8	0	1	3	1	3.9
2	2	6	0	0	3	0	1	2	9	4	2.7
3	3	7	0	0	8	1	7	2	1	6	3.5
4	4	4	1	4	1	1	7	2	4	0	2.8
5	31	7	2	7	1	0	4	7	1	4	6.4
6	7	3	7	4	4	4	1	1	0	1	3.2
7	8	2	8	3	0	6	0	9	1	0	3.7
8	9	2	9	1	1	4	4	24	0	2	5.6
9	0	1	0	4	0	0	3	0	7	16	3.1
10	10	0	1	7	3	0	0	7	2	5	3.5
Mean											3.84
Kentucky Wonder:											
1	0	8	3	9	2	7	7	0	1	34	7.1
2	1	4	6	8	7	8	1	3	0	7	4.5
3	3	9	2	1	5	10	3	4	3	0	4.0
4	8	6	4	16	0	9	4	1	6	4	5.8
5	0	1	4	0	1	7	2	0	7	9	3.1
6	9	3	0	0	0	6	0	1	2	8	2.9
7	3	4	7	8	0	2	1	5	1	0	3.1
8	0	1	3	4	0	1	3	6	0	1	1.9
9	1	0	4	3	7	7	4	0	3	2	3.1
10	1	3	7	1	0	8	7	3	6	4	4.0
Mean											3.95

Inasmuch as these data indicated that the root knot resistance of Alabama No. 1 bean is not due to the lack of entry or differences in the rate of entry of nematode larvae it appeared desirable to extend the experiment to include a number of varieties and species of plants of known resistance and susceptibility. Thirty varieties and species were studied, including the two beans used in the previous experiment. Counts were made of the number of nematode larvae in rootlets, using

[7] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 4, 302 pp., illus. Edinburgh and London. 1932.

the same technique as previously described with the exception that the nematode inoculum used was prepared from badly diseased roots of common beans, lima beans, tomatoes, and cowpeas rather than common beans alone. The mean number of larvae observed in the rootlets of each species is presented in table 2. Considerable variation within species occurred as indicated by the relatively high standard-error values.

TABLE 2.—*Mean number of nematode larvae observed in rootlets of 30 varieties and species of plants*

Response [1] to root knot and scientific name	Common name	Variety name	Total rootlets studied	Mean larvae per rootlet [2]
Resistant:			*Number*	*Number*
Phaseolus vulgaris L.	Common bean	Alabama No. 1	28	4.6±1.3
P. lunatus L.	Lima bean	Hopi 155	30	5.0±1.0
Vigna sinensis (Torner) Savi.	Cowpea	Conch	21	5.8±1.2
Do.	do	Iron	33	4.5± .8
Do.	do	Brabham	32	7.2±1.6
Do.	do	Victor	30	3.5± .9
Crotalaria spectabilis Roth.	Crotalaria		38	3.9± .7
Raphanus sativus L.	Radish	Scarlet Globe	23	5.1±1.1
Brassica japonica Siebold	Mustard	Giant Southern Curled	20	3.9± .9
B. rapa L.	Turnip	Purple Top White Globe	37	3.7± .5
Allium cepa L.	Onion	Prizetaker	26	4.5±1.3
A. vineale L.	Wild onion		20	4.1±1.0
Cosmos bipinnatus Cav.	Cosmos	Early White	21	3.5±1.1
C. sulphureus Cav.	Golden cosmos	Orange Flare	26	5.8±1.5
Zinnia elegans Jacq.	Zinnia	Lilliput Salmon Rose	29	6.3±1.1
Secale cereale L.	Rye	Abruzzi	30	4.8±1.2
Avena sativa L.	Oat	Hasting Hundred Bushel	33	3.4± .7
Triticum aestivum L.	Wheat	Alabama Bluestem	27	4.0±1.3
Zea mays L.	Corn	Trucker Favorite	20	5.4±1.2
Do.	do	Golden Bantam	21	4.9±1.5
Sorghum vulgare Pers.	Sorghum	Sagrain	22	5.6±1.1
Sorghum halepense (L.) Pers.	Johnson grass		23	4.7±1.4
Cynodon dactylon L.	Bermuda grass		20	4.1± .8
Digitaria sanguinalis (L.) Scop.	Crabgrass		26	4.6±1.2
Mean				4.7± .2
Susceptible:				
P. vulgaris L.	Common bean	Kentucky Wonder	30	4.5±1.2
Do.	do	Burpee Stringless Greenpod	23	7.8±2.4
P. lunatus L.	Lima bean	Henderson Bush	29	4.8±1.2
Vigna sinensis (Torner) Savi.	Cowpea	Cream Crowder	23	5.8±1.4
Lycopersicum esculentum Mill.	Tomato	Marglobe	26	6.2±1.7
Hibiscus esculentus L.	Okra	Hasting Dwarf	22	4.1±1.2
Mean				5.4± .6

[1] The classification into resistant and susceptible varieties is based on observations of the writer and on various reports in the literature. Those species which show any appreciable signs of resistance are included in the resistant class.

[2] The standard-error values were calculated on an individual-variety basis. Standard-error values for the means of the resistant and susceptible varieties are for the mean of a series of means.

It will be seen from the data presented in table 2 that, in the species studied, no general relationship exists between the rate of larval entry and root knot resistance. Although the mean number of larvae observed in rootlets of susceptible species is slightly greater than that observed in rootlets of resistant species, there is no significant difference between these means at the 5-percent level.

There are no significant differences at the 1-percent level between the mean number of larvae observed in the rootlets of any two varieties or species. At the 5-percent level the mean for Brabham cowpea appears significantly higher than that for Victor cowpea and Hasting Hundred Bushel oats; however, no importance can be attached to this

in view of the fact that all three are somewhat resistant to root knot. Furthermore, the high mean of Brabham cowpea is due to a few rootlets containing an exceptionally large number of larvae.

The question was raised whether the resistant varieties and species would develop galls if allowed to grow beyond the seedling stage in soil as severely infested as that used in these experiments. In order to investigate this point 10 seedlings each of a number of varieties studied in the preceding experiment were transplanted to heavily inoculated soil in a greenhouse bed. All seedlings used for this purpose were grown in the same soil and at the same time as those from which rootlets were removed for the preceding study. The resistant varieties tested in this way were Alabama No. 1 bean, Hopi 155 lima bean, Conch cowpea, rye, oats, and Trucker Favorite corn. Three susceptible varieties, Kentucky Wonder bean, Cream Crowder cowpea, and tomato, were included for purposes of comparison. Roots were examined 7 weeks after transplanting, and in every instance the extent of root-knot development agreed with numerous field observations. Many galls were found on the three susceptible varieties, and some galls of a small size were observed on Hopi 155 lima bean. A few very small galls were found on roots of Conch cowpea and Trucker Favorite corn, while rye and oats showed no evidence of nematode infestation with the exception of a few very slight swellings. Inasmuch as rootlets from other seedlings of these varieties grown at the same time and under exactly the same conditions showed no significant differences in the rate of larval entry, these differences in the development of root knot must be due to hereditary differences in ability to resist gall formation.

In order to determine whether resistance to the entry of nematode larvae might develop with age adult plants of a few resistant and susceptible varieties growing in pots were sunk in soil that had been thoroughly inoculated with nematodes. The roots were allowed to to grow through the bottom of the pot. Tips 1 cm long were then removed and stained as in the previous experiments. Twenty rootlets each of three susceptible and three resistant varieties were observed.

The mean number of larvae found in rootlets of each variety is recorded in the following tabulation:

	Variety	Mean number of larvae per rootlet [1]
Resistant:		
	Alabama No. 1 bean	7. 1±4. 5
	Hopi 155 lima bean	3. 0± . 7
	Trucker Favorite corn	3. 1±1. 2
Susceptible:		
	Kentucky Wonder bean	4. 3±1. 2
	Henderson Bush lima bean	2. 7± . 7
	Marglobe tomato	4. 0± . 9

[1] The standard-error values were calculated on a single-variety basis.

It will be seen that as many larvae entered the rootlets of adult plants of Alabama No. 1 bean, Hopi 155 lima bean, and Trucker Favorite corn, three resistant varieties, as entered those of Kentucky Wonder bean, Henderson Bush lima bean, or Marglobe tomato, all of which are highly susceptible. It is interesting to note that one rootlet of Alabama No. 1 observed in connection with this study contained so many nematode larvae it was impossible to count them accurately. The estimated number was 90. A portion of this rootlet is shown in plate 1, *C*.

An experiment was conducted in order to determine whether Hopi 155 lima bean developed the ability to resist gall formation with age. Henderson Bush lima was used for purposes of comparison. Seeds of each variety were planted in 2½-inch pots in nematode-free soil on June 1, June 28, and July 12. The first two plantings were shifted to 4-inch pots when 2 weeks old. On July 26 the pots were lifted and sunk in inoculated soil, and on the same day seeds of both varieties were planted in the same inoculated soil in a greenhouse bench. Thus there were seeds and 2-, 4-, and 8-week-old plants all in the same lot of inoculated soil. Roots grew through the bottoms of all the pots within 4 days after setting. Therefore, roots of the different-aged plants including the seedlings were exposed to nematodes at approximately the same time. Roots of plants of different ages were examined from time to time, and all were removed 8 weeks after being exposed to nematode-infested soil.

The following observations were made during the course of this experiment on root knot development in Hopi 155 and Henderson Bush lima bean.

Three-week-old seedlings of the two varieties showed the same degree of root knot development, confirming earlier observations of the writer.[8]

Young roots of both varieties, including those which grew from seedlings and potted plants of various ages, developed root knot to the same degree.

As infected roots grew, the galls on Henderson Bush lima developed at a more rapid rate than those on Hopi 155.

At the end of 8 weeks roots of Henderson Bush lima had much larger galls than roots of Hopi 155. Galls on the former had coalesced to some extent, and many of them had begun to decay.

Galls on Hopi 155 were similar in size to those commonly found on the same variety in the field. For the most part they were nothing more than slight swellings and were never more than twice the diameter of the root on which they were borne. There were no signs of decay.

DISCUSSION AND CONCLUSIONS

Although the subject has not been discussed to any extent in the literature because of the lack of experimental investigations, it has probably been assumed by many workers that root knot resistance is due to a plant's ability to prevent the entrance of nematode larvae. Steiner[9] states: "In the study of plant-parasitic nemas, the resistance of a plant means that it opposes the entrance of the nemas by some mechanical or perhaps chemical means." Although Arzberger[10] did not state such a conclusion, it is quite evident that he assumed the morphological root characteristics he found correlated with resistance to nematode infestation actually prevented the entry of nematode larvae. Insofar as the writer can determine no concrete evidence that larvae do not enter the roots of resistant varieties has been presented.

The experiments summarized in tables 1 and 2 show that root knot resistance, in the representative varieties and species studied, is not

[8] BARRONS, KEITH C. See footnote 3.
[9] STEINER, G. See p. 527 of citation mentioned in footnote 4.
[10] ARZBERGER, E. G. See footnote 5.

due to the lack of entry of nematode larvae. No significant differences were noted between the rate of entry in the most resistant and in susceptible plants in the seedling stage. Even rootlets of onion, which has been said to be repellant to nematodes, contained as many larvae as other species. That no ability to resist entry is developed with age is indicated by the experiment summarized in the tabulation on page 267.

From the observations made on root knot development on roots of Henderson Bush and Hopi 155 lima beans at different ages it appears that the resistance of the latter is only partial in the adult plant, although it is very definitely resistant to a notable degree when compared with Henderson Bush. This confirms numerous field observations made by the writer. Although seedlings and young roots on adult plants of Hopi 155 appear fully as susceptible as similar roots of Henderson Bush, gall development seems to be arrested as the roots mature. The fact that galls on the two varieties are the same size in the seedling stage but later are much smaller on Hopi 155 indicates that something within the roots of this variety retards root knot development.

It is hoped that further investigations on the comparative morphology and physiology of resistant and nonresistant varieties and species will make possible a better understanding of this problem. It seems reasonable to conclude on the basis of the data presented in this paper that resistance is due to factors entirely within the plant itself that do not exclude the entrance of larvae but either (1) kill the nematode after it enters or (2) cause it to starve. Nematologists are generally agreed that nematodes feed on the contents of the giant cells the formation of which is apparently induced by salivary secretions of the nematode itself. In view of the adverse conditions which nematodes have been known to withstand in experiments it seems more likely that their lack of development in resistant plants is a matter of starvation rather than death by some lethal agent. Although it is not impossible that certain morphological root characteristics accomplish or contribute to resistance, it seemed more reasonable to postulate resistance due to certain physiological factors.

As a speculative hypothesis the writer suggests that resistance may be due to certain chemicals within the roots of resistant plants that counteract or neutralize the giant-cell-inducing effect of the salivary secretions of the nematode. It is believed possible to account for the known facts concerning resistance with such a hypothesis. For example the occurrence of varietal differences in cowpeas could be thus explained. Isbell [11] has found that Conch is highly resistant to root knot, showing only occasional galls, while Cream Crowder is very susceptible. Between these two extremes are varieties possessing various degrees of resistance. It seems possible that these varietal differences may be due to their varying ability to synthesize the hypothetical neutralizing substances. Conch has been observed to form an occasional root knot gall. This variety may synthesize these substances in quantities sufficient to neutralize the secretions of practically all larvae. However, if an enormous number of larvae entered the root at one point, it is possible that neutralizing substances would not be present in quantities sufficient to counteract their com-

[11] ISBELL, C. L. NEMATODE INJURY TO THE ROOTS OF TABLE VARIETIES OF COWPEAS. Ala. Agr. Expt. Sta. Ann. Rept. 45: 29-30. 1934.

bined effect. Under such circumstances giant cells would be formed, but the resulting gall would never attain the size of those on susceptible varieties formed under the same circumstances because of the continual synthesis of the neutralizing substances. This same explanation may account for the occurrence of occasional galls on a number of species that are generally considered resistant.

This hypothesis conforms with the observed facts of adult plant resistance. As previously mentioned, seedlings and young roots of adult plants of Hopi 155 lima bean grown in nematode-infested soil develop root knot to the same degree as Henderson Bush lima while galls on mature roots of the former variety are much smaller and do not decay as readily. It is possible that the neutralizing substances are lacking in the young roots of Hopi 155 but are synthesized as the roots mature, thus inhibiting the development of the nematodes and thereby reducing the extent of gall formation. According to this hypothesis Henderson Bush lima and other highly susceptible plants lack the ability to synthesize these substances. Some plants show few, if any, signs of gall formation in the seedling stage. As mentioned earlier in this paper, only occasional slight swellings are found on the rootlets of Alabama No. 1 bean. This observation can fit into the neutralizing-substance concept by postulating that such varieties possess the ability to synthesize these substances in relatively large quantities at an early age.

The results of these experiments raise the question whether the phenomenon of host specialization in the root knot nematode as discussed by Steiner [12] involves differences in ability to perceive and enter the root or in ability to induce giant-cell formation once the larvae have entered. It is believed that most workers have held the former concept, but it seems possible that variations in the response of different nematode populations to the same plants may be due to differences in the salivary secretions of the nematode larvae. Possibly the gradual adaptation of a population to a specific host is a matter of genetic changes in the chemical nature of its secretions rather than changes in ability to perceive and enter the host. It is hoped that this fundamental problem which is of interest both to breeders and nematologists will be solved by further investigations.

The results of the above experiments indicate that the use of highly susceptible plants as trap crops may be unnecessary. If as many larvae enter resistant plants in which the nematodes will not go through their life cycle, why are not such plants just as effective in reducing nematode populations as the highly susceptible crops that will permit the population to increase unless properly handled? Although theoretically sound, the trap-crop method of nematode control has not proved practical, partly because of the necessity of plowing under or removing the infested plants before the first life cycle had been completed. In the light of these investigations there appears to be no reason why resistant varieties may not be used as trap crops. The resistant crops that have been used in rotations for the purpose of reducing nematode populations have probably been acting as traps.

[12] STEINER, G. See footnote 4.

SUMMARY

A microscopical study was made of rootlets from seedlings of Alabama No. 1 and Kentucky Wonder beans. It was found that as many larvae entered the rootlets of the former variety, which is highly resistant, as of the latter, which is susceptible to root knot.

A further microscopical study of the rootlets of a number of susceptible and resistant varieties and species of plants indicated that there were no significant differences in the rate of larval entry either in the seedling or adult stage. It had previously been assumed by a number of workers that resistant plants actually resisted the entry of nematode larvae.

Hopi 155 lima has been observed to be somewhat resistant to root knot in the field, but it was found that roots of seedlings of this variety developed root knot to the same degree as seedlings of Henderson Bush lima when grown in artificially inoculated soil. Experimental observations on plants of different ages indicated that young roots of Hopi 155 were susceptible regardless of the age of the plant on which they were growing. However, as the roots matured, the galls on Henderson Bush developed at a more rapid rate. Galls on Hopi 155 never grew very large and were less subject to decay than those on the roots of susceptible lima beans.

The hypothesis is proposed that root knot resistance is due to substances synthesized by the plant that counteract the giant-cell-inducing effect of the salivary secretions of nematode larvae. Such a hypothesis accounts for the known facts concerning hereditary resistance.

The results of these experiments raise two questions of fundamental importance: (1) Whether the phenomenon of host specialization may not be due to genetic differences in the chemical nature of salivary secretions and the resulting ability to induce giant-cell formation rather than differences in ability to perceive and enter the host; and (2) whether resistant plants may not be superior to susceptible plants as trap crops, inasmuch as equal numbers of larvae appear to enter the roots of resistant plants but do not reproduce.

EFFECT OF TEMPERATURE OF DIGESTION, CHEMICAL COMPOSITION, AND SIZE OF PARTICLES ON PRODUCTION OF FUEL GAS FROM FARM WASTES[1]

By G. H. NELSON, *cooperative agent*, ROBERT P. STRAKA, *associate bacteriologist*, and MAX LEVINE, *cooperative agent*, *Industrial Farm Products Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

Research on the anaerobic decomposition of farm waste for the production of methane and other gases for use as fuel on the farm has been stimulated by the development of a method of treating sewage in which the settleable organic solids are digested anaerobically by bacteria. Large quantities of gas having a relatively high calorific value are produced by this method. There are many difficulties, however, in the application of this sewage-sludge digestion process to fibrous farm wastes. Their tendency to lie close to or on the surface of liquid media in which they are suspended introduces a particularly difficult mechanical problem, and their low nitrogen content, in contrast to sewage sludges, makes it necessary to provide additional nitrogen.

Boruff and Buswell (*4, 7 8*)[2] reported the production of about 5 cubic feet of gas per pound of cornstalks, about 4.8 cubic feet per pound of flax straw, 3.4 cubic feet per pound of flax shives, 10.8 cubic feet per pound of filter paper, 5.8 cubic feet per pound of banana skins and stems, 3.9 cubic feet per pound of wheat straw, and 4.8 cubic feet per pound of newspaper.

Hussey, Row, and Allison (*9*), in a study of the utilization of cellulosic wastes in conjunction with sewage for the purpose of augmenting the gas supply, report but 1.5 cubic feet of gas per pound of peanut hulls, 3.9 cubic feet per pound of leaf waste, and 10.7 cubic feet per pound of wastepaper added to the sewage sludge.

The purpose of the investigation reported in this paper was to ascertain the effect of (1) temperature of digestion, (2) chemical composition, and (3) size of particles on the rate and quantity of gas produced from fibrous farm wastes by anaerobic microbial decomposition.

MATERIALS EMPLOYED

Particular attention was paid to cornstalks, wheat straw, artichoke tops, and seed flax straw. The cornstalks were used in the form of flour and as chopped material. The wheat straw was used as ground (1 mm) and as chopped material. In addition to these typical farm wastes filter paper and alkali lignin obtained from corncobs were employed in a few experiments to ascertain the digestibility of prepared cellulose and prepared lignin, two constituents characteristic of the wastes in question. Another substance used, referred to as "cook liquor," was the liquid residue left after digesting cornstalks with

[1] Received for publication September 12, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 286.

alkali for the purpose of manufacturing wallboard. This liquid residue was evaporated to the consistency of a heavy paste, when it took on the appearance of blackstrap molasses. Cook liquor was included in the investigation because it was thought that the information obtained might be of use in the problem of treating wastes from wallboard factories should this industry develop to a point where numerous small plants would be located in the farm areas.

PROCEDURE

In general the procedure for preparation of an active seed was to add a little cornstalk flour to a mass of partly digested sewage sludge and allow digestion to go practically to completion, after which addi-

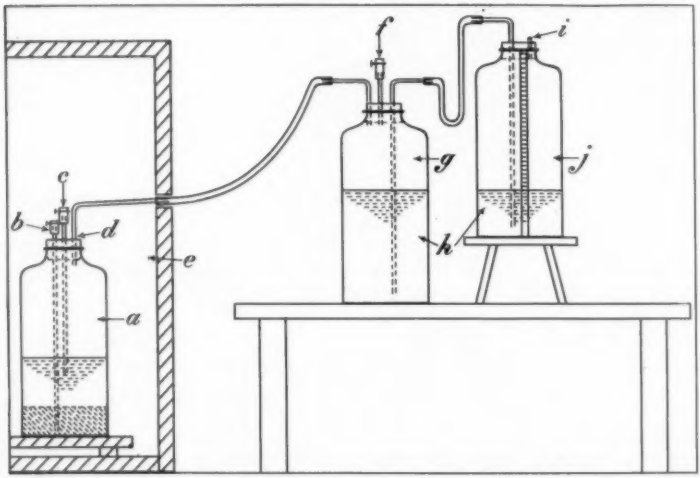


FIGURE 1.—Fermentation apparatus: *a*, Digestion bottle; *b*, tube for removing samples; *c*, tube for adding chemicals; *d*, tube to gas collector; *e*, incubator; *f*, tube for gas samples; *g*, gas-collecting bottle; *h*, saturated salt solution; *i*, air vent; *j*, bottle for measuring gas.

tional quantities of cornstalk flour were added. After several such additions the digested material was used as the seed for the various experiments.

To a given volume of seed in a suitable container was added a sufficient quantity of test material to give the desired ratio of freshly added volatile solids [3] to volatile solids in the seed. This ratio was approximately 1 to 1 except in a few experiments at the high temperature (50°–55° C.), where the amount of volatile solids added was increased until the ratio was approximately 2.5 to 1. The ratios of fresh volatile solids to volatile solids in the seed for each of the materials studied are shown in table 2.

Each material was tested at digestion temperatures of 28° to 30° C. and 50° to 55°. The two series might be referred to as the mesophilic and thermophilic series, respectively.

[3] The term "volatile solids" as used throughout this paper refers to the material lost when materials were heated in a furnace at 600° C.

The volume of the fermenting mixtures ranged from about 1,000 to 5,500 cc. As no significant differences in rate of gas production due to differences in volume were observed, the volumes are not recorded in the tables.

The digestion vessels consisted of wide-mouth glass bottles fitted with three-hole rubber stoppers arranged as shown in figure 1. Through one of these holes a ½-inch glass tube extended down to within a few inches of the bottom of the liquid. This was used for removing small samples from time to time to determine the change in reaction. A second tube, which extended through the stopper into the liquid, was used for the addition of ammonium salts and other reagents when necessary. The third tube which extended just through the rubber stopper, connected the digestion bottle with the bottle for the collection of gas.

In the experiments with filter paper and artichoke tops lime was added several times after the fermentation had started in order to prevent excessive acidity. The total nitrogen content in all these substances was low, running from zero in the filter paper to 0.68 percent in the cook liquor. If not already in the seed, an adequate amount of nitrogen was added in the form of ammonium phosphate. The total nitrogen (as ammonia and organic nitrogen) in the fermenting mixture ranged from 500 to 2,500 parts per million.

The analyses of the various materials employed are shown in table 1.

TABLE 1.—*Analyses of farm wastes used*

Material	Moisture [1]	Total solids [1]	Volatile solids	Ash [1]	Pentosans [2]	Cellulose [3]	Lignin [4]	Other material	Ash [1]	Total N_2 [1]
	Percent	*Percent*	*Percent*	*Percent*	*Percent* [5]	*Percent* [5]	*Percent* [5]	*Percent* [5]	*Percent* [1]	*Percent* [1]
Lignin	3.07	96.93	96.87	0.06	0.758	0	87.37	11.81	0.07	0.529
Filter paper	2.78	97.22	97.22	0	0	100.00	0	0	0	0
Cook liquor	56.91	43.09	37.02	6.07	28.58	0	24.10	33.22	14.10	.68
Cornstalk flour	5.22	94.78	86.90	7.88	29.72	33.55	18.49	9.93	8.31	.269
Artichoke-top flour	6.36	93.64	90.25	3.39	14.74	22.50	11.54	47.60	3.62	.435
Chopped cornstalks	3.46	96.54	90.15	6.39	26.92	34.90	18.55	13.02	6.61	.32
Chopped wheat straw [6]	4.71	95.29	88.20	7.09	28.60	33.60	15.37	14.96	7.47	.329
Ground wheat straw [6]	5.53	94.47	88.04	6.43	27.65	34.05	18.92	12.63	6.75	------
Seed flax straw	5.38	94.62	89.46	5.16	19.97	49.55	21.65	3.37	5.46	------

[1] Analysis by American Public Health Association and American Water Works Association (*2*).
[2] Association of Official Agricultural Chemists method (*3, p. 344*).
[3] Norman-Jenkins method (*18*).
[4] Method of Bray (*6*), as modified by Peterson, Walde, and Hixon (*13*).
[5] On dry basis.
[6] The samples of chopped and ground wheat straw were taken from different batches. When the nature of the material, possible varieties, and different harvesting methods are taken into consideration, divergencies in composition of the magnitude in this table are not large.

It will be noticed from table 1 that the cook liquor and artichoke-top flour were particularly high in constituents other than pentosans, cellulose, and lignin. These constituents are for the most part water soluble and probably are carbohydrates. The percentages of pentosans and cellulose in the wheat straw and cornstalks were practically the same. The seed flax straw, on the other hand, differed strikingly from the other fibrous farm wastes in that it contained considerably less pentosans but more cellulose.

RATE AND QUANTITY OF GAS PRODUCED BY MATERIALS TESTED

The results obtained in the various experiments are given in table 2 and shown graphically in figure 2. The relation of temperature to digestion and the rate of gas production for the various substances tested are shown in figure 3. The results obtained with each substance will be discussed briefly.

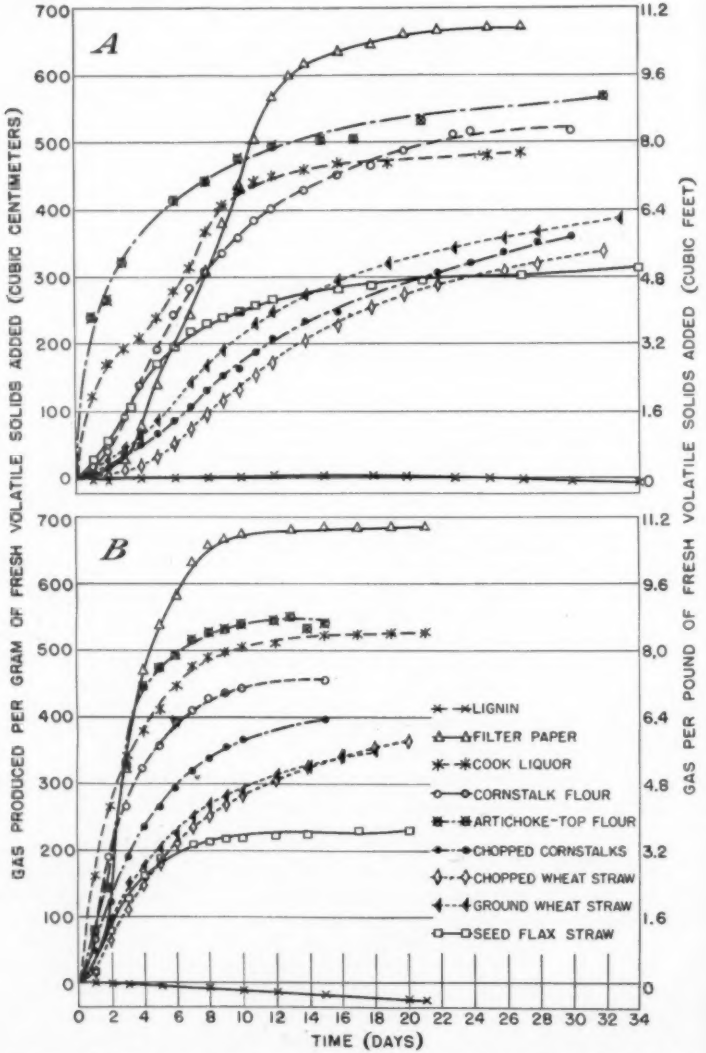


FIGURE 2.—Gas from farm wastes digested at: *A*, 28° to 30° C; *B*, 50° to 55°.

LIGNIN

The lignin used was the so-called alkali lignin prepared from corncobs. The authors hoped to develop a flora that would be particularly effective in attacking lignin, but as may be seen from the results both at the low temperature (28° to 30° C.) and at the high temperature (50° to 55°), gas was not obtained when lignin was added to a ripe and active seed. As shown in table 2, the total amount of gas produced in the lignin-seed mixture was actually less than that pro-

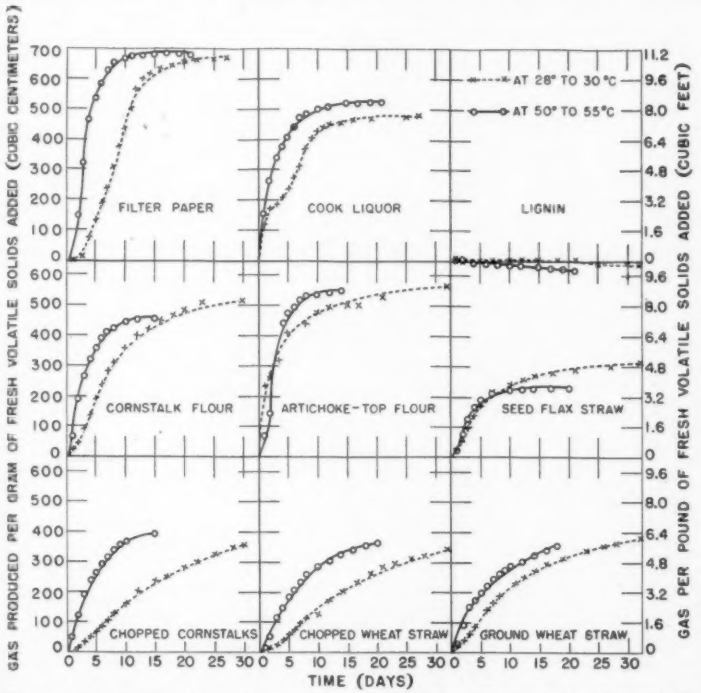


FIGURE 3.—Comparison of gas production at 28° to 30° and at 50° to 55° C.

duced from the seed control. It is evident that under the conditions of these experiments the digestion of lignin did not produce gas to any appreciable extent. The gas produced was low in methane (16.5 to 19.7 percent) and very high in hydrogen (16.4 to 16.6 percent). The hydrogen figures in this and the other series are probably higher than they would be in large-scale operation because of the temporary aerobic condition at the beginning of fermentation.

FILTER PAPER

A high-grade Whatman quantitative filter paper was used in the experiments. Two runs were made at each of the temperatures. During one of the runs at the lower temperature repeated additions of small quantities of paper were made. The volume of gas produced in 27 days at 28° to 30° C. was 674 cc per gram of filter paper added.

TABLE 2.—*Gas from farm wastes digested at 28° to 30° and at 50° to 55° C.*

28° TO 30° C.

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added																		
				1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days	14 days	15 days	16 days	17 days	18 days	19 days
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Lignin (alkali) [1]	36–70	0.84–1.06	4	−1.8	−2.4	0.7	1.3	1.4	1.6	2.2	2.6	2.3	4.5	4.9	4.6	4.4	------	4.0	3.6	------	4.2	3.5
Filter paper	36	.85	2	.2	------	18.1	78.0	138.0	194.3	240.4	310.3	378.4	438.7	500.9	568.0	601.0	618.0	------	633.0	637.6	645.3	658.9
Cook liquor	38.15	.79	2	120.0	171.8	189.4	207.3	239.4	278.8	314.0	371.8	405.4	430.4	441.9	450.4	454.6	457.9	------	469.3	------	------	471.0
Cornstalk flour	25–100	.85–1.06	6	24.1	40.7	90.5	140.2	194.4	243.4	284.2	312.8	335.8	359.1	383.2	403.0	416.9	428.3	441.8	451.0	459.6	469.0	474.7
Artichoke-top flour	25	1.09	2	238.0	266.2	321.8	------	------	413.3	------	444.9	------	478.5	------	495.3	------	------	503.7	505.9	------	------	------
Chopped corn-stalks	25–75	1.05	2	4.8	15.6	37.3	50.4	67.7	87.4	108.0	130.1	151.4	163.6	186.8	206.6	221.6	230.6	239.6	246.1	252.7	262.5	272.3
Chopped wheat straw	69–100	1.01–1.04	3	------	11.1	15.3	24.9	36.4	57.8	78.6	98.2	116.9	135.3	157.8	175.8	------	208.4	------	233.4	243.9	------	263.2
Ground wheat straw	71–100	1.01–1.04	4	------	29.3	46.9	65.4	88.4	------	141.9	165.8	191.2	------	232.8	250.4	------	276.1	------	294.3	------	------	316.3
Seed flax straw	100	1.01	2	22.5	54.4	94.4	137.8	171.9	199.4	220.8	232.3	242.0	249.8	258.4	264.2	------	274.0	------	281.1	287.1	------	------

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added															Gas produced per gram of total solids added	Composition of gas		
				20 days	21 days	22 days	23 days	24 days	25 days	26 days	27 days	28 days	29 days	30 days	31 days	32 days	33 days	34 days		CO_2	H_2	CH_4
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Per cent*	*Per cent*	*Per cent*
Lignin (alkali) [1]	36–70	0.84–1.06	4	2.9	3.8	------	2.6	------	−0.4	------	−1.1	------	------	−2.4	------	−3.9	------	−5.8	−5.79	13.3	16.6	19.7
Filter paper	36	.85	2	662.7	------	664.5	------	------	669.0	------	673.6	------	------	------	------	------	------	------	673.6	38.5	4.2	49.4
Cook liquor	38.15	.79	2	479.3	------	------	------	------	479.0	------	483.6	------	------	------	------	------	------	------	416.0	32.2	3.7	59.3
Cornstalk flour	25–100	.85–1.06	6	487.3	493.4	------	512.1	516.0	------	------	------	------	------	517.9	------	------	------	------	475.0	38.5	4.4	53.7
Artichoke-top flour	25	1.09	2	------	533.1	------	------	------	------	------	------	------	------	------	------	572.5	------	------	551.5	38.2	8.6	49.1
Chopped corn-stalks	25–75	1.05	2	282.0	293.1	304.3	312.3	320.4	328.3	336.1	343.9	351.9	355.8	359.7	------	------	------	------	336.0	32.4	8.8	53.9
Chopped wheat straw	69–100	1.01–1.04	3	------	280.1	------	295.6	------	------	312.2	------	322.6	------	------	------	342.7	------	------	317.2	36.0	6.2	55.4
Ground wheat straw	71–100	1.01–1.04	4	------	------	------	341.8	------	------	356.3	------	367.8	------	------	------	------	388.5	------	362.5	36.5	4.7	55.3
Seed flax straw	100	1.01	2	------	292.9	295.1	------	------	------	------	305.3	------	------	------	------	------	------	316.9	300.0	37.4	1.1	58.8

50° TO 55° C.

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added												
				1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days	13 days
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Lignin [1] (alkali)	32.1–116.0	0.88–1.10	4	1.09	8.57	2.3	−1.13	−2.58	−5.10	−7.8	−10.6	−11.9	−13.3	−15.2	−15.3	−16.4
Filter paper	20	1.06	2	7.9	150.2	320.1	469.8	537.4	587.8	631.7	655.5	666.2	674.5	678.7	-------	679.8
Cook liquor	20	.95	2	160.0	264.2	340.4	379.8	411.6	444.7	475.6	487.4	498.0	507.6	-------	511.2	515.2
Cornstalk flour	12.4–127.0	.97–1.30	8	69.1	193.2	269.1	324.4	359.7	397.9	416.8	428.2	436.8	446.9	-------	454.0	454.6
Artichoke-top flour	23.3	.50	2	71.9	142.9	324.2	448.6	477.2	493.5	520.6	530.3	531.8	539.7	544.1	545.1	528.9
Chopped cornstalks	25.0–48.7	1.02–1.35	4	51.0	124.2	194.0	239.8	268.6	296.7	322.8	342.1	360.5	369.7	-------	-------	-------
Chopped wheat straw	14.5–165.0	1.00–2.58	3	18.6	69.4	116.2	148.3	184.8	211.5	238.4	261.7	273.6	285.8	-------	309.0	322.2
Ground wheat straw	15.0–30.0	1.03–2.58	3	31.3	96.8	154.6	175.3	202.8	222.6	247.4	266.2	277.8	288.4	297.1	305.8	313.7
Seed flax straw	25	2.05	3	21.8	78.2	129.7	166.6	191.2	-------	211.5	216.2	220.2	222.9	222.9	223.6	225.7

Material	Quantity used	Ratio of volatile solids added to volatile solids in seed	Runs	Gas produced per gram of volatile solids added								Gas produced per gram of total solids added	Composition of gas		
				14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days		CO_2	H_2	CH_4
	Grams		*Number*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Percent*	*Percent*	*Percent*
Lignin [1] (alkali)	32.1–116.0	0.88–1.10	4	−17.8	−18.9	−20.2	−21.6	−22.1	−23.0	−23.9	−29.9	−29.8	21.5	16.4	16.5
Filter paper	20	1.06	2	-------	684.6	-------	687.0	-------	687.4	-------	687.7	687.7	40.3	.7	55.7
Cook liquor	20	.95	2	-------	521.9	-------	525.2	-------	527.2	-------	528.5	454.0	34.5	1.9	56.8
Cornstalk flour	12.4–127.0	.97–1.30	8	-------	455.9	-------	-------	-------	-------	-------	-------	419.0	32.4	10.3	53.5
Artichoke-top flour	23.3	.50	2	538.6	-------	-------	-------	-------	-------	-------	-------	519.0	28.1	11.0	52.6
Chopped cornstalks	25.0–48.7	1.02–1.35	4	-------	395.7	-------	-------	-------	-------	-------	-------	369.5	35.5	3.6	55.9
Chopped wheat straw	14.5–165.0	1.00–2.58	3	328.1	334.8	341.1	348.5	354.1	360.8	364.8	-------	338.0	29.9	8.8	54.1
Ground wheat straw	15.0–30.0	1.03–2.58	3	327.0	335.9	343.4	-------	354.5	-------	-------	-------	331.0	28.1	7.0	57.3
Seed flax straw	25	2.05	3	227.0	-------	-------	230.3	231.0	231.0	231.0	-------	218.5	27.1	2.7	55.7

[1] Minus sign indicates that less gas was produced from the lignin and the seed than from the seed alone.

This is equivalent to 10.8 cubic feet of gas per pound of filter paper added. Ninety percent of the gas was produced, however, in 14 days (*1, 2, 3, 4, 6, 8*).

At the high temperatures the rate of gas production was considerably greater; a total of 688 cc per gram of filter paper added, or 11 cubic feet per pound, was produced in 21 days. At this temperature 90 percent of the gas was produced in 6.8 days, or about half the time in which a similar quantity of gas was produced at 28° to 30° C.

For the purpose of comparing the effect of temperature on the rate of gas production, the time required to produce a definite volume, 300 cc per gram of volatile solids added, at each temperature range was determined for each material. The time required for filter paper to produce this volume of gas was 7.8 days at 28° to 30° C. and about 2.9 days at 50° to 55°. The higher temperature reduced the digestion time 63 percent.

The mixture had a marked tendency to become acid. This was particularly true at the low temperature.

The gas contained approximately 49.4 percent of methane at the low temperature and 55.7 percent at the higher temperature. The hydrogen content was 4.2 and 0.7 percent, respectively. Here, again, it is desired to point out that in large-scale operation the methane content would probably rise to 55 to 60 percent.

COOK LIQUOR

Two runs of the cook liquor were made at each temperature. During one of the low-temperature runs repeated additions of small quantities of material were made. During a digestion period of 27 days at 28° to 30° C., 484 cc of gas per gram of volatile solids added was produced; 90 percent of the gas was produced in approximately 11 days. At a temperature of 50° to 55° the volume of gas produced in 21 days was 529 cc per gram of volatile solids added, 90 percent of this gas being produced in 7 days. The volume of gas produced per pound of volatile solids added as cook liquor was 7.6 cubic feet at 28° to 30° and 8.45 cubic feet at 50° to 55°.

The time required to produce 300 cc of gas per gram of volatile solids added was 6.5 days at the lower temperature and 2.5 days or 62 percent less, at the higher temperature.

The gas produced contained 59.3 percent of methane at the lower temperature and 56.8 percent at the higher temperature. There was no significant difference in the nature of the gas evolved at the two temperatures.

ARTICHOKE TOPS

Artichoke tops were ground to a flour, and two runs were made at each of the temperatures. As in the previous series, one of the runs at the lower temperature consisted of repeated additions of small quantities of artichoke-top flour.

At 28° to 30° C., 573 cc of gas per gram of volatile solids added was produced in 32 days, and at 50° to 55°, 539 cc was produced in 14 days. This corresponds to 9.2 and 8.6 cubic feet per pound of volatile solids added at the low and high temperatures, respectively. Ninety percent of the gas was produced at the lower temperature in 19 days and at the higher temperature in about 6 days. The period for production of 300 cc of gas per gram of volatile solids added was

2.4 days at 28° to 30°, as compared with 2.5 days at 50° to 55°. This is in marked contrast with the results in the filter-paper and cook-liquor experiments, where the higher temperature resulted in much more rapid production of gas.

The gas evolved contained 49.1 percent of methane at 28° to 30° C. and 52.6 percent at 50° to 55°.

CORNSTALKS

The cornstalk series included six runs with cornstalk flour and two runs with chopped cornstalks at 28° to 30° C., and eight runs with cornstalk flour and four runs with chopped cornstalks at 50° to 55°.

The cornstalk flour, which had a fineness of approximately 200 mesh, was made by grinding cornstalks in a rod mill. The chopped cornstalks, which passed a ½-inch screen but were retained by a ¼-inch screen, were cut in a silage shredder.

The volume of fermenting materials in the various runs ranged from 1,500 to 5,500 cc, but there were no significant differences in the rate of gas production. In the cornstalk-flour series at the lower temperature 518 cc of gas per gram of volatile solids added was produced in 30 days, as compared with 456 cc in 15 days at 50° to 55° C. This corresponds to 8.3 cubic feet per pound of volatile solids at 28° to 30° and 7.3 cubic feet at 50° to 55°. Ninety percent of the gas was produced in a little more than 17 days at the lower temperature and in somewhat less than 7 days at the higher temperature. The time required to evolve 300 cc of gas was 7.5 days at 28° to 30° and 3.5 days at 50° to 55°, or approximately 53 percent less at the higher temperature.

The gas evolved at 28° to 30° C. contained 53.7 percent of methane, as compared with 53.5 percent at the higher temperature.

The rate of gas production by the chopped cornstalks was very much slower. At 28° to 30° C., 360 cc of gas per gram (5.76 cubic feet per pound) of volatile solids added was produced in 30 days, while at 50° to 55°, 396 cc per gram (6.3 cubic feet per pound) was produced in 15 days. At the lower temperature 90 percent of the gas evolved was produced in 24.5 days; at the higher temperature the same percentage of gas was produced in about 8.5 days.

The time required for the production of 300 cc of gas per gram of volatile solids added was 22 days at the lower temperature and 6 days at the higher temperature, or approximately 73 percent less at the higher temperature. The gas contained 53.9 percent of methane in the experiments at 28° to 30° C. and 55.9 percent in the 50° to 55° series.

Figure 3 shows the influence of size of the cornstalk material on the rate of digeston at the two temperatures. It will be noted that the chopped cornstalks and the cornstalk flour were decomposed rapidly at the higher temperature, the time required to produce 300 cc of gas being 6 days for the chopped material and 3.5 days for the flour. The time required for the chopped cornstalks was not quite twice that required for the flour. At the lower temperature the chopped cornstalks required 22 days and the flour 7.5 days to produce this quantity of gas. At this temperature nearly three times as long a digestion period was required for the chopped cornstalks as for the cornstalk flour. It is suggested that the difference in the relative

rates of gas production by the chopped cornstalk and the flour at the two temperatures was probably due to an increased rate of penetration of the enzymes into the chopped material at the higher temperature.

The results also indicate that the effect of temperature depends not only on the chemical composition of the material undergoing digestion, for the two materials were practically identical chemically, but also on its physical character, as has been mentioned by Norman (*11*).

WHEAT STRAW

The wheat straw series consisted of straw that had been chopped or ground by means of a hammer mill. The chopped straw consisted of particles about one-quarter of an inch long and the ground straw of particles about one twenty-fifth of an inch in diameter.

It will be noted from table 2 that in some of the experiments at 50° to 55° C. the quantity of volatile solids added as wheat straw was more than two and a half times that in the seed, whereas at the lower temperature the ratio was always 1 to 1. It was thought that this larger proportion of fresh volatile solids might have slowed up the rate of gas production at the higher temperature, but examination of the individual runs disclosed that the rate of gas production was not slower in those experiments in which the larger proportion of volatile solids was employed.

In 28 days' digestion at 28° to 30° C. the gas produced per gram of volatile solids added was 323 cc for the chopped wheat straw and 368 cc for the ground wheat straw. This corresponds to a gas volume of 5.2 and 5.9 cubic feet, respectively, per pound. At the higher temperature the volumes of gas produced by the chopped and ground materials in 18 days were almost identical, 354 and 355 cc, which corresponds to 5.7 cubic feet per pound of volatile solids added. Ninety percent of the gas evolved was obtained at the lower temperature in 24 to 25 days and at the higher temperature in approximately 13 to 14 days.

The ground wheat straw produced 300 cc of gas per gram of volatile solids added in approximately 16.5 days at the lower temperature and in 11.5 days at the higher temperature. The digestion period was decreased about 30 percent by employing the higher temperature.

For chopped wheat straw the time required for the production of 300 cc of gas was approximately 25 days at 28° to 30° C. and 11 days at 50° to 55°. In this case the higher temperature decreased the digestion period about 56 percent. The effect of temperature on the rate of digestion was more marked with the chopped than with the ground material. This was also noted in the cornstalk series.

The amount of methane in the gas was approximately the same in all the series, 55.3 and 55.4 percent being present in the ground and chopped straw series, respectively, at 28° to 30° C. and 57.3 percent and 54.1 percent at 50° to 55°.

SEED FLAX STRAW

The volume of gas produced by the seed flax straw was somewhat higher at the lower temperature. The rates of gas production were almost identical at the two temperatures for the first 8 days of digestion, after which there seemed to be no appreciable increase in gas

at the higher temperature, a volume of 216 cc after 8 days increasing to only 231 cc after 20 days. Apparently some change took place which stopped fermentation after 8 days. At the lower temperature, however, the slow evolution of gas continued so that a volume of 232 cc after 8 days rose to 293 cc in 21 days and 317 cc in 34 days. The total amount of gas produced per gram of volatile solids added was very much smaller in the seed flax straw than in any of the other substances tested except lignin, being only 317 cc per gram (5.1 cubic feet per pound) after 34 days at 28° to 30° C. and only 231 cc per gram (3.7 cubic feet per pound) after 20 days at 50° to 55°. Of the total gas produced, 90 percent was evolved in about 16.5 days at the lower temperature, whereas at the higher temperature only about 6.5 days were required for the same proportion of gas. The time required for the evolution of 200 cc of gas per gram of volatile solids added was about 6.5 days at each of the two temperatures.

The quantity of methane present was 58.8 percent at 28° to 30° C. and 55.7 percent at 50° to 55°.

DISCUSSION

In general, the materials tested fall into four distinct groups on the basis of the rate of gas production and the quantity of gas produced per gram of material added. This is very well shown in figure 2, particularly in *A*.

One group, which includes filter paper, artichoke-top flour, cornstalk flour, and cook liquor, is characterized by a high rate of gas production and a large volume of gas per unit of material added. Each of these substances produced 450 cc or more of gas per gram of volatile materials added (7.2 cubic feet per pound). At 28° to 30° C. each of these substances produced 200 cc of gas per gram (3.2 cubic feet per pound) of volatile material added in 6 days or less and 300 cc per gram (4.8 cubic feet per pound) in less than 8 days. At 50° to 55°, 400 cc or more of gas per gram of volatile solids added (6.4 cubic feet per pound) was produced in approximately 6 days or less, 300 cc (4.8 cubic feet per pound) in less than 4 days, and 200 cc (3.2 cubic feet per pound) in about 2 days.

The second group of materials includes chopped wheat straw, ground wheat straw, and chopped cornstalks. This group is characterized by moderate gas production (350 to 385 cc per gram of volatile material added at 28° to 30° C. and about 325 cc at 50° to 55°) and a rate of gas production distinctly lower than that in the first group. Thus at 28° to 30° it required 9 to 14 days for the production of 200 cc and 17 to 22 days for 300 cc of gas per gram of volatile solids added. At 50° to 55°, 200 cc of gas was produced in 3 to 5 days; 300 cc was produced in 11 days by the wheat staw and in 6 days by the cornstalks.

The seed flax straw is in a group by itself. The rate of gas production at the low temperature was intermediate between the first two groups, but the total volume of gas produced was distinctly less than that in either group. At the higher temperature the rate of gas production was about the same as that in the second group, but the total gas produced was very much less.

Lignin is also in a class by itself in that there is no evidence that it was decomposed with the production of gas, a phenomenon that has been previously reported by Levine et al. (*10*), Boruff and Buswell (*5*), and Acharya (*1*).

The question arises as to the reasons for the different rates of decomposition of the various substances tested, and whether these differences might be correlated with the chemical composition of the materials. The substances in the first group, characterized by a rapid rate of gas production and a large quantity of gas, (1) were entirely free from lignin (filter paper); or (2) had a high content of carbonaceous materials other than pentosans, cellulose, and lignin (cook liquor and artichoke-top flour); or (3) consisted of very small particles (cornstalk flour). The high rate of gas production and large volume of gas in the first three subtsances are therefore attributed to the comparatively large quantities of carbonaceous materials other than lignin and cellulose in the cook liquor and artichoke-top flour and to the homogeneous character and lack of lignin in the filter paper The rapid fermentation of the cornstalk flour (in contrast to the chopped cornstalks) is attributed to the small size of the particles and consequent large surface exposure.

The rate of decomposition of fibrous material subjected to anaerobic digestion is influenced by a number of factors, among which are temperature, size of particles subjected to decomposition, and chemical composition of the material.

A comparison of the results obtained with cornstalks and wheat straw indicates, however, that factors other than chemical composition (content of cellulose, lignin, and pentosans) and the size of particles are significant with respect to rate of decomposition at various temperatures. Table 3 shows the time required for the production of 300 cc of gas per gram of volatile matter added as chopped cornstalks, cornstalk flour, chopped wheat straw, and ground wheat straw.

TABLE 3.—*Time required for production of 300 cc of gas per gram of volatile matter in cornstalks and wheat straw*

Temperature (° C.)	Cornstalks		Wheat straw	
	Chopped	Flour	Chopped	Ground
	Days	*Days*	*Days*	*Days*
28–30	22	7.5	25	16.5
50–55	6	3.5	11	11.5

It will be noted that the time required for the production of 300 cc of gas at 28° to 30° C. was approximately the same for the chopped cornstalks and the chopped wheat straw and that at 50° to 55° the digestion periods were almost identical for the chopped and for the ground wheat straw. In view of the fact that the chopped wheat straw was very much smaller than the chopped cornstalks and the ground wheat straw was smaller than the chopped wheat straw and considering further that the chemical composition of all these substances was practically the same, it is apparent that factors in addition to size and chemical composition must be concerned with the differences in rates of decomposition observed. If the rate of decomposition depended merely on size and chemical content, the smaller chopped wheat straw should have decomposed more readily than the chopped cornstalks at the lower temperature, and the finer ground wheat straw more rapidly than the chopped wheat straw at the higher temperature. A comparison of the chopped wheat straw and chopped cornstalks at

the higher temperature, emphasizes further that size alone does not account for the differences observed in rates of decomposition. Here it will be noted that the chopped cornstalks required 6 days for the production of 300 cc of gas, while the chopped wheat straw, which was smaller and should therefore presumably have been attacked more readily if size alone were the determining factor, required 11 days. The effect of temperature on the rate of decomposition was appreciably different with the chopped cornstalks and chopped wheat straw. With the former the period for the production of 300 cc of gas dropped from 22 to 6 days as the temperature of digestion was raised from 28°–30° to 50°–55°; with the latter it dropped from 25 to 11 days.

It is suggested that the physical structure may mechanically protect the fermentable pentosans and celluloses of some materials against biological action. This concept might explain the difference in the rate of decomposition of different size particles of the same material. Another explanation is that the chemical combination of some fermentable substances prevents them from being readily available to micro-organisms. This suggestion might explain why the chopped cornstalks decomposed more rapidly than the wheat straw, which was distinctly smaller.

It will be noticed from figure 3 that the effect of temperature is much more marked on the chopped cornstalks than on the cornstalk flour. This indicates that fermentable substances which are mechanically protected from bacterial action are made more readily available to micro-organisms and their enzymes by the exposure resulting from comminution. Mechanical protection is presumably more effective at the lower temperature than at the higher temperature, where the rate of diffusion of enzymes is considerably greater.

In the experiments with seed flax straw no appreciable difference was observed in the rate of gas production as a result of the change in temperature. This material happened to be particularly high in cellulose though distinctly lower in pentosans than the cornstalks and wheat straw, but the volume of gas produced was markedly smaller than that produced by any of the other substances except lignin. The total pentosans and cellulose amounted to approximately 69 percent in the seed flax straw and approximately 62 percent in the cornstalks and wheat straw. The small amount of gas produced by the seed flax straw indicates that the fermentable substances were in some manner not readily available to microbial action. A considerable portion of this material consisted of shives, which are not readily fermentable, although analysis indicates that their content of cellulose, pentosans, and lignin does not differ appreciably from that in other materials. This is another indication that the content of these three constituents is not an index of the fermentability of a substance.

Apparently either mechanical protection or chemical linkage of the pentosans, cellulose, and other constituents markedly influences the digestibility and rate of decomposition of plant materials. Consequently, at present a fermentation test is the only available means for determining the susceptibility of a substance to microbial decomposition for the production of fuel gases.

The fact that the effect of temperature on the rate of digestion of different materials tested was not in all cases the same (in some in-

stances gas production being three times as rapid at 50° to 55° C. as at 28° to 30°, while in others no significant differences were shown) emphasizes the importance of testing each material at various temperatures. The results obtained with one farm waste evidently cannot be applied to another as far as the effect of temperature on rate of anaerobic production of gas is concerned.

SUMMARY AND CONCLUSIONS

The rate and quantity of gas produced by the anaerobic decomposition of chopped cornstalks, chopped wheat straw, ground wheat straw, cornstalk flour, filter paper, seed flax straw, artichoke-top flour, cook liquor from wallboard manufacture, and alkali lignin were determined at 28° to 30° and 50° to 55° C.

The effect of temperature on the rate of anaerobic decomposition was different for the various fibrous farm wastes tested. Thus, with artichoke-top flour and seed flax straw, the rate of digestion at 28° to 30° C. was not appreciably different from that at 50° to 55°. With filter paper and cook liquor the same quantity of gas (300 cc per gram of volatile solids) was produced in about one-third the time at 50° to 55°. With cornstalk flour and chopped wheat straw it required approximately half as long to produce 300 cc of gas at 55° as at 28° to 30°. With ground wheat straw, the digestion time was reduced about one-third by employment of the higher temperatures, while with chopped cornstalks only about one-quarter of the digestion period was necessary at 50° to 55° as at 28° to 30°, to produce the same amount of gas. In view of the fact that the chemical composition of these substances is almost identical, it is apparent that composition cannot be used as a basis for predicting the probable influence of temperature on the rate of digestion.

The effect of temperature on the rate of digestion of fibrous farm wastes is more marked with coarser materials, such as chopped cornstalks and chopped wheat straw, than with finer materials, such as cornstalk flour and ground wheat straw.

The chemical composition of fibrous wastes (their content of lignin, cellulose, and pentosans) is not a dependable basis for evaluation of ease of anaerobic decomposition or of the rate and quantity of gas produced per unit weight of material.

Physical factors play an important role in the susceptibility of fibrous material to anaerobic microbial decomposition.

At present a fermentation test under controlled conditions is the only means of ascertaining the rate and quantity of gas obtainable from a fibrous farm waste by anaerobic microbial decomposition.

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INACTIVATION OF PYRETHRUM AFTER INGESTION BY THE SOUTHERN ARMYWORM AND DURING INCUBATION WITH ITS TISSUES[1]

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INTRODUCTION

Pyrethrum is widely used as a contact insecticide, but it has not been found generally effective as a stomach poison. In experiments with larvae of the southern armyworm (*Prodenia eridania* (Cram.)) the writer has found that, although they are killed by pyrethrum applied externally, they are apparently not affected after ingestion of the substance. Furthermore, the feces of these larvae failed to show the presence of the toxic principles of pyrethrum when tested against mosquito larvae. The purpose of this investigation was to determine whether the toxic principles of pyrethrum are inactivated, in whole or in part, after ingestion by the southern armyworm, and the kind of tissues or the conditions that might be responsible for such inactivation.

Swingle (*10*)[2] found that, although larvae of the imported cabbage worm (*Pieris rapae* L.) are rapidly affected and killed by pyrethrum applied externally, they are not killed after ingestion of the substance. He further demonstrated[3] that, even though the larvae appear to suffer no ill effects, the toxic principles do not pass through the insect unchanged, for feces of larvae that had ingested pyrethrum were not toxic when applied as a contact dust to other larvae of the same species. Swingle suggested that the action of the digestive juices apparently had destroyed the toxicity.

Audiffren (*3*) obtained a chemical reaction indicating the presence of chrysanthemum monocarboxylic acid in the urine of human patients to whom pyrethrum had been administered as an anthelmintic. This indicates the cleavage of pyrethrin I.

MATERIALS

The larvae used in these experiments were sixth instars of the southern armyworm, reared in a greenhouse insectary on turnip plants and cut lettuce.

The unextracted pyrethrum used was powdered Kenya pyrethrum flowers having a pyrethrin I content of 0.53 percent and a pyrethrin II content of 0.64 percent, as determined by the Seil method.[4] A petroleum ether extract of pyrethrum was also used in one experiment.

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[1] Received for publication August 3, 1938.

[2] Italic numbers in parentheses refer to Literature Cited, p. 294.

[3] Unpublished report, 1934.

[4] This analysis was made by S. I. Gertler, of the Division of Insecticide Investigations of this Bureau.

Toxicity was determined by the use of mosquito larvae (*Culex fatigans* Wied.) as biological indicators, according to the method reported by Campbell and associates (*5, 9*). This method has been used successfully for the determination of comparative toxicity of a number of insecticidal substances, including pyrethrum, to which mosquito larvae are very sensitive.

INACTIVATION AFTER INGESTION BY SOUTHERN ARMYWORM LARVAE

Since there is evidence (*8, 14*) that the toxic principles may be absorbed directly through the body integument, it appears not unlikely that they could be absorbed through the gut wall. The fact that southern armyworm larvae are unaffected after ingestion of pyrethrum, and that the feces are nontoxic to mosquito larvae, indicates that the toxicity has been destroyed either within the gut or within the tissues after absorption through the gut wall.

PROCEDURE

In this experiment southern armyworm larvae were fed pyrethrum, and later all the tissues of the larvae as well as the gut contents and feces were tested against mosquito larvae for the presence of the toxic principles.

The pyrethrum was fed to the larvae in sandwiches prepared according to the method of Campbell and Filmer (*4*). Disks 22 mm in diameter were cut from turnip leaves and dusted with pyrethrum by the air-float method under a large battery jar. Each disk received approximately 1 mg of the dust. The sandwiches were then prepared, starch paste being used as the adhesive. Each sandwich was placed in a 150-ml beaker with one larva that had been deprived of food for several hours and retained there for 18 hours overnight. In the morning those larvae that had ingested entire sandwiches and had not regurgitated more than a negligible amount were conserved on fresh turnip leaves for 6 hours longer. All the feces excreted during the 24-hour period, and also the washings of the beakers in which the larvae had been conserved, were collected and combined. The blood of each larva was obtained by snipping an abdominal leg, and the blood of all the larvae was combined. The larvae were then dissected, the following different kinds of materials were isolated, and like materials from all the larvae were combined and ground with sand: Skin, tissue of the digestive tract and the malpighian tubules, fat body, muscular and all other tissues, contents of the foregut and hind-gut, and contents of the midgut. The different kinds of materials prepared as outlined above from larvae that had ingested pyrethrum will be referred to as tests to distinguish them from the checks, which were prepared at the same time.

Three checks were used. The first was designed to demonstrate the effect of the larval tissues alone on the mortality of the mosquito larvae, the second the combined effects of larval tissue and pyrethrum, and the third the effect of pyrethrum alone. For the first and second checks the tissue preparations were made from larvae that had received the same treatment as those used for the tests except that the

sandwiches ingested did not contain pyrethrum. For the second check pyrethrum was mixed with the tissues while they were being ground. For the third check microscope cover glasses of the same diameter as the leaf disks were dusted with pyrethrum when the leaf disks were dusted, so that the quantity of pyrethrum would be the same as that ingested by the larvae.

The tests and checks were diluted with distilled water. The exact concentration of the pyrethrum in the suspensions as prepared is unknown, as the quantity of dust on the leaf disks could not be determined with accuracy. However, the exact strength is immaterial to the present investigation. The pyrethrum-only check was prepared in such concentration that it gave 100-percent morbidity or mortality of the mosquito larvae within 1 hour. The proper concentration to use was obtained by preliminary trials. The check consisting of larval materials to which pyrethrum had been added was prepared in the same concentration. The tests and the checks consisting of the various larval materials without pyrethrum were prepared 100 times as concentrated as the pyrethrum-only check. The morbidity or mortality of the mosquito larvae in so strong a concentration should indicate the presence of even greatly reduced quantities of the toxic principles in the materials from larvae that had ingested pyrethrum.

One hundred mosquito larvae were used in each concentration of each test and check. The mortality was determined after 1 hour and after 20 hours at 29° C. in the dark.

RESULTS

All the mosquito larvae in the pyrethrum-only check and in the pyrethrum-added check were dead or moribund within 1 hour. In the tests and in the no-pyrethrum check the mortality was negligible even though the concentration was 100 times that of the pyrethrum-only check and the period of exposure was 20 hours. Similar results were obtained on two repetitions.

The results of this experiment indicate that 6 to 24 hours after ingestion little, if any, of the toxic principles of pyrethrum are present in any of the tissues of larvae that have ingested pyrethrum, in the contents of the digestive tract, or in the feces. It is concluded, therefore, that the toxic principles of pyrethrum are inactivated, either wholly or in high degree, after ingestion by the southern armyworm.

INACTIVATION BY THE ISOLATED TISSUES OF SOUTHERN ARMYWORM LARVAE

To identify the tissues responsible for any possible inactivation of the toxic principles of pyrethrum, an experiment was made to determine whether the toxicity would be destroyed or altered by incubation with one or more of the isolated tissues.

Since the pyrethrins occur chiefly within the cell walls in the achenes of pyrethrum flowers, they are not immediately exposed to the action of their environment even in fine powder. A slight reduction in toxicity of unextracted pyrethrum powder that was noted in unreported preliminary experiments might have been due to the breakdown of only that small portion of the pyrethrins that was liberated

in grinding. A petroleum ether extract of pyrethrum was therefore used in addition to the unextracted pyrethrum.

PROCEDURE

The blood, fat body, skin and muscle, digestive tract, and gut contents from healthy larvae that had not ingested pyrethrum were isolated, and like tissues were combined and ground with sand. In one series of tests unextracted pyrethrum suspended in distilled water in the proportion of 1 mg per milliliter was added at the rate of 0.5 mg per larva, and in another series a petroleum ether extract of pyrethrum suspended in distilled water in the proportion of 0.16 mg per milliliter was added at the rate of 0.08 mg per larva.

Two checks were used. The first check was designed to demonstrate the effects of the larval tissues alone on the mortality of the mosquito larvae, and was made with tissue preparations corresponding to those used in the tests, to which water, not containing pyrethrum, but equivalent in amount to the pyrethrum suspension, was added. The purpose of the second check was to demonstrate the effect of pyrethrum alone, and was made with an amount of pyrethrum equivalent to that mixed with the tissues in the tests, suspended in water.

The preparations were incubated in the dark for 18 hours at 29° C. The material used in each series was divided into two parts. One part was allowed to stand in shallow vessels during the incubation. Since unreported experiments had demonstrated that a considerable quantity of the toxic principles settles out of suspension during this period, resulting in their unequal distribution in the tissue preparations, the other part was agitated in a mechanical shaker throughout the incubation period.

Toxicity was determined by the use of mosquito larvae as described for the first experiment. The pyrethrum-only check was prepared in such concentration that it gave 100-percent morbidity or mortality of mosquito larvae within 2 hours. Concentrations one-fifth, one twenty-fifth, and one-fiftieth this strength were also prepared. The tests and the no-pyrethrum check were prepared in the same concentrations.

RESULTS

The effect of the larval materials alone on the mortality of the mosquito larvae in the tests may be disregarded, since the mortality in the checks containing larval material but no pyrethrum was negligible even after 20 hours. With both unextracted and extracted pyrethrum the mosquito larvae in the checks containing highly concentrated pyrethrum were dead or moribund within 2 hours. In the lowest concentration the mortality was low even after 20 hours. The degree of reduction in toxicity of the toxic principles of both the unextracted and the extracted pyrethrum during incubation with various tissues and with the gut contents of southern armyworm larvae was estimated by comparing the mortality of the mosquito larvae in these tests with that in the pyrethrum check that was incubated in water without larval materials. The results are given in table 1.

TABLE 1.—*Reduction in toxicity of unextracted and extracted pyrethrum during incubation with various tissues and with the gut contents of southern armyworm larvae, as indicated by the mortality of mosquito larvae*

Tissue	Degree of reduction in toxicity			
	Not agitated		Agitated	
	Unextracted	Extracted	Unextracted	Extracted
Blood	Little or none	Little or none	Little or none	Partial?
Fat body	Partial	Great	Very great	Very great.
Skin and muscle	do	Partial	Partial	Great.
Digestive tract	Little or none	do	Little or none	Partial.
Gut contents	do	do	do	Partial?

The extracted pyrethrum underwent slightly greater reduction in toxicity than the unextracted material, and agitation throughout the incubation period increased the reduction in toxicity of both extracted and unextracted pyrethrum.

With the possible exception of the agitated extracted material, neither extracted nor unextracted material was appreciably reduced in toxicity by incubation with blood. The toxicity of unextracted pyrethrum was not appreciably reduced by incubation with either the tissue or the contents of the digestive tract. The toxicity of extracted pyrethrum was partly reduced by incubation with these materials, but the degree of reduction was not increased by agitation. Both extracted and unextracted pyrethrum were reduced in toxicity by incubation with skin and muscle tissue, and the reduction of extracted pyrethrum was increased by agitation. The toxicity of unextracted pyrethrum was partly reduced, and that of extracted pyrethrum greatly reduced, when incubated with fat body and not agitated. Very great reduction occurred with both extracted and unextracted material when agitated during incubation.

DISCUSSION

The high degree of inactivation of pyrethrum which has been shown to occur after ingestion by the southern armyworm and during exposure to different tissues of this insect may be explained by the fact that the pyrethrins I and II, the toxic principles of pyrethrum, are unsaturated keto esters and chemically unstable. A great variety of chemical and physical conditions exist in the insect and in insect tissues that, singly or combined, might influence the break-down of the pyrethrins. The possible effect of hydrolytic enzymes, such as esterases, on the decomposition of the pyrethrins has been suggested (*2*).

It is well known that pyrethrum may lose insecticidal value in storage and that it is affected by heat, moisture, light, and other natural conditions (*1, 6, 7, 13, 15*). Tattersfield (*11*) and Tattersfield and Martin (*13*) have shown that pyrethrum powders and dusts lose their insecticidal activity on exposure to light and air, and that such loss of activity is retarded by the incorporation of certain antioxidants with the pyrethrum-dust preparations. Tattersfield and Hobson (*12*) found pyrethrum extracts to be comparatively stable in solution or suspension in certain solvents in closed vessels and in the presence of various emulsifiers.

The decrease in toxicity of pyrethrum during incubation with certain tissues of armyworm larvae suggests that some of these tissues or their products may be responsible for the inactivation which occurs in living larvae. Since the two conditions are quite different, no definite conclusion can be drawn. It is doubtful that the tissues or secretions of the digestive tract are alone responsible. The fat body alone probably could inactivate the pyrethrins after their liberation from the cell walls in the achenes and their absorption into the hemolymph.

SUMMARY AND CONCLUSIONS

An investigation was undertaken to determine whether the toxic principles of pyrethrum are inactivated, in whole or in part, after ingestion by the southern armyworm (*Prodenia eridania* (Cram.)), and also to learn the kind of tissues that might be responsible for such inactivation. To study the first problem the pyrethrum was fed to the larvae in turnip-leaf sandwiches, and later the tissues, gut contents, and feces were ground and tested against mosquito larvae for the presence of toxic principles. To study the second problem the pyrethrum was mixed with the various tissues and the gut contents from healthy larvae and incubated at 29° C. in the dark for 18 hours. Half the material was agitated during incubation. The material was then tested against mosquito larvae for the presence of toxic principles.

The results indicate that the toxic principles of pyrethrum may become inactivated, wholly or in high degree, after ingestion by the southern armyworm. The tissue of the digestive tract and the gut contents may reduce the toxicity of unextracted pyrethrum little or none, and of extracted pyrethrum only partially. The blood may have little or no effect on the toxicity. The skin and muscle tissues may greatly reduce the toxicity of agitated extracted pyrethrum, but only partially reduce the toxicity of unagitated extracted pyrethrum and of either unagitated or agitated unextracted pyrethrum. The fat body may reduce the toxicity of unextracted pyrethrum partially and of extracted pyrethrum greatly, even when these materials are not agitated, but the toxicity of both when agitated with fat body during incubation may be very greatly reduced.

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CYTOGENETIC OBSERVATIONS IN LACTUCA[1]

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INTRODUCTION

The work on *Lactuca* reported in this paper was initiated with a twofold purpose: (1) To make a survey of meiosis in certain species and (2) to define the limits of interspecific hybridization in order to determine the possibility of introducing desirable genes of other species into cultivated lettuce (*Lactuca sativa* L.).

REVIEW OF LITERATURE

Recently Babcock, Stebbins, and Jenkins (*1*) [3] have reviewed the pertinent literature. These authors have reported the chromosome numbers of 25 species of *Lactuca*. Of the species of *Lactuca* the chromosome number of which is known, 11 have 8 pairs, 12 have 9 pairs, and 6 are presumably amphidiploids with 17 pairs of chromosomes. The latter are endemic American species and are thought to have originated from crosses between species with eight and nine chromosomes, followed by doubling of the entire complement.

A paper by Cooper and Mahony (*2*) is also of interest. In a study of meiosis in *Lactuca scariola* var. *integrata*, they found that the plants examined were structural hybrids with seven bivalents and a ring of four chromosomes. Data on fertility and condition of the pollen were not given, hence there is no means of judging the physiological effects of the translocation. In earlier work by Gates and Rees (*5*) with *L. sativa* 50 percent of the first metaphase plates showed less than the characteristic haploid number of nine chromosomes. Thus it appears likely that they also were dealing with plants in which translocation had occurred.

There are some reports of attempted species crossing in the genus. Ernst-Schwarzenbach (*4*) made reciprocal pollinations, using *Lactuca scariola* and a number of cultivated varieties of *L. sativa*. The majority of these pollinations were successful and resulted in viable seed. A few reciprocal pollinations were made between *L. scariola* and *L. virosa;* none set seed. Negative results were obtained in 12 further crossings in which *L. virosa* was used as a pollen and seed parent with 10 varieties of *L. sativa*.

Durst (*3*) has crossed several varieties of *Lactuca sativa* with *L. scariola*. From data secured in F_2 and subsequent generations he concluded that differences between the two species are largely accounted for by mutations of dominant genes.

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[2] The authors gratefully acknowledge the assistance of the Division of Plant Exploration and Introduction in supplying much of the material for the investigation here reported. Thanks are also extended to Dr. G. L. Stebbins, Jr., Division of Genetics, University of California, for identifying the material.
[3] Italic numbers in parentheses refer to Literature Cited, p. 306.

MATERIALS AND METHODS

The species of *Lactuca* studied by the authors were assembled at the United States Horticultural Field Station, La Jolla, Calif. The following is a list of the species and varieties, with the sources from which they were obtained:

Lactuca sativa var. Imperial D	Jagger.
Lactuca sativa var. Chinese Stem	P. E. and I.,[1] from China.
Lactuca sativa var. Bavarian Brown	P. E. and I., from Europe.
Lactuca sativa	P. E. and I., from India.
Lactuca scariola var. *integrata* Gren. and Godr.	Collected at Woodland, Calif.
Lactuca scariola L.	P. E. and I., from Union of Soviet Socialist Republics.
Lactuca scariola var. *angustana* Hort.	P. E. and I., from Sweden.
F_1 (Imperial 152 × *L. scariola*)	Jagger.
Lactuca chondrillaeflora Bor.	P. E. and I., from France.
Lactuca perennis L.	P. E. and I., from England.
Lactuca tartarica (L.) C. A. Meyer	P. E. and I., from India.
Lactuca cretica Desf.	P. E. and I., from Union of Soviet Socialist Republics.
Lactuca bourgaei Boiss.	P. E. and I., from England.
Lactuca canadensis L.	J. B. Norton, Hartsville, S. C.
Lactuca graminifolia Mich.	Do.

The cytological observations were for the most part made on acetocarmine smears of meiotic chromosomes. In some cases the counts were checked by root-tip studies.

The preparation of the slides of *Lactuca* material was rather difficult, and it was found necessary to develop a special technique for their preservation as permanent smears. Since the technique differs in several ways from the ordinary methods of permanent smear preparation, it will be described here in some detail. Flower heads in which the pollen mother cells are actively dividing are found by making a temporary acetocarmine smear of an individual floret. The remaining florets are fixed in a mixture of acetic acid and absolute alcohol (1 part glacial acetic acid, 2 parts absolute alcohol). They are allowed to remain in this mixture for 2 to 3 hours. The fixative is then drained off and replaced with absolute alcohol, in which the material is permitted to stand overnight. This procedure seems to harden the delicate pollen mother cells; consequently they are not easily crushed in the smearing process. The individual flowers are placed on a slide in a drop of acetocarmine, and the anthers are dissected out and crushed. The extraneous material is removed, and the cover slip added. By heating gently, and with slight pressure on the cover slip, it is possible to cause the cells to adhere either to the slide or to the cover slip. Heating also promotes greater differentiation between chromosomes and cytoplasm.

The cover is floated off in a mixture of equal parts of absolute alcohol and acetic acid. Both slide and cover are transferred through several changes of 95-percent alcohol and allowed to remain 15 minutes or longer in the latter solution. Slide and cover slip are then reunited by mounting in either euparal or diaphane. This step should be carried out in an atmosphere of low humidity, and care should be exercised to avoid breathing on the slide lest the preparation become fogged and unsuitable for observation. By

[1] Division of Plant Exploration and Introduction, Bureau of Plant Industry, U. S. Department of Agriculture.

mounting directly in euparal or diaphane, immersion in the higher alcohols and xylols becomes unnecessary. This is a point of considerable importance, since the latter two fluids, especially xylol, are prone to distort and darken the cytoplasm. Material prepared according to the above schedule has proved very satisfactory. Such preparations are particularly suitable for purposes of photomicrography, since the cells are flattened and the entire chromosome complement is brought into one plane.

In the hybridization work, the usual "depollination" method of Oliver (7) has been followed. This consists in washing the pollen from the expanded stigmas by means of a fine stream of water. The stigmas are then dried and dusted with pollen from the desired plants. Protection from insect contamination after pollination seems to be unnecessary.

OBSERVATIONS

The information obtained as to chromosome number, morphology of chromosome complement, and pollen fertility has been recorded in table 1. The letters L, M, and S refer to the relative lengths of the chromosomes within a genom. The percentage of pollen fertility was determined by counts from acetocarmine smears of mature anthers.

MEIOSIS IN THE NINE-CHROMOSOME SPECIES

In all varieties of *Lactuca sativa* the nine bivalents pair regularly (fig. 1, *A*). Three are definitely longer than the remaining members of the complement, and three are much shorter; thus the relationship may be expressed by the ideogram 3L+3M+3S. This seems to be valid for all varieties of *L. sativa* examined. An occasional irregularity may occur when plants are subjected to abnormally high temperatures during meiosis. Such a case is shown in figure 1, *B*, where one bivalent has separated somewhat in advance of the others. It is doubtful whether a disturbance of this nature would cause the production of sterile pollen, and it must be concluded that in its chromosome behavior *L. sativa* is a very stable species.

TABLE 1.—*Chromosome number, ideogram, and pollen fertility in species of Lactuca*

Species	Chromosomes	Ideogram [1]	Pollen fertility
	Number		*Percent*
Lactuca sativa var. Imperial D	9	3L+3M+3S	90–95
L. sativa var. Chinese Stem	9	3L+3M+3S	90
L. sativa var. Bavarian Brown	9	3L+3M+3S	90
L. sativa (from India)	9		90–95
L. scariola var. *integrata* (from California)	9	4L+2M+3S	80–85
L. scariola (from Union of Soviet Socialist Republics)	9	4L+2M+3S	90
L. scariola var. *augustana*	9	4L+2M+3S	90
F_1 (Imperial 152 × *L. scariola*)	9	3L+3M+3S	90
L. chondrillaeflora	9	2L+7M	85–90
L. perennis	9	7M+2S	90
L. tartarica	9	3L+6M	90
L. cretica	8	4L+4M	90–95
L. bourgaei	8	8M	80–85
L. canadensis	17		90–92
L. graminifolia	17		85–90

[1] Relative length of chromosomes is indicated by the initial letters of the words "long," "medium," and "short."

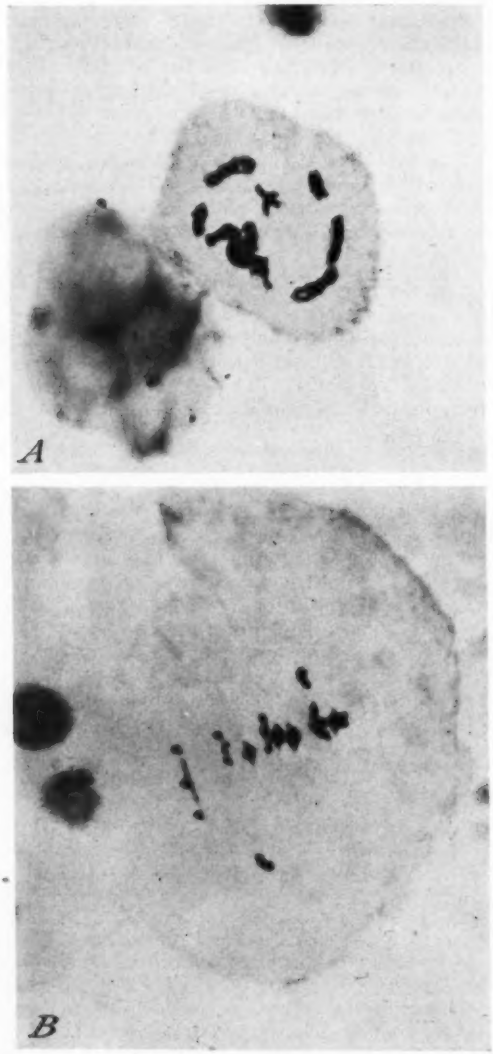

FIGURE 1.—Photomicrographs showing chromosomes of cultivated lettuce (*Lactuca sativa*): *A*, *L. sativa* var. Imperial D, diakinesis (× 1,100); *B*, *L. sativa* var. Imperial D, metaphase, profile view, showing one bivalent dividing precociously (× 820).

The nine chromosomes of *Lactuca scariola* are morphologically very similar to those of *L. sativa* (fig. 2, *A*). A close study, however, indicates that there is not the extreme differentiation in size of the individual chromosomes within the set that is found in *L. sativa*. There seem to be four bivalents of somewhat greater length than the remainder, and three that are somewhat shorter. The complement of *L. scariola* may therefore be represented by the ideogram 4L+2M+3S. A number of strains and varieties of *L. scariola* from widely scattered geographic ranges have been examined and no differences in the morphology of the chromosome complement have been found.

In spite of possible slight differences in the make-up of their genoms, fertile F_1 hybrids in which the chromosomes pair regularly at meiosis (fig. 2, *B*) can be obtained easily between varieties of *Lactuca scariola* and *L. sativa*. The percentage of viable pollen is approximately the same as in the parent species. There are some indications of hybrid vigor in the F_1, but these are not striking. The fact that varieties of *L. sativa* and *L. scariola* can be crossed at will, with the production of fertile offspring, is of very real importance. Strains that are resistant to mildew (*Bremia* sp.) and that promise to become commercially acceptable varieties have been selected from original crosses of this type.

Lactuca chondrillaeflora.—This species has inconspicuous yellow flowers, and the unusual habit of shedding its leaves with the initiation of the flowering cycle. There are nine chromosomes, two of which are definitely longer than the remainder (fig. 2, *C*). Meiosis is regular, and pollen fertility high (85 to 90 percent). Attempts to hybridize this species with *L. sativa* and the tetraploids *L. canadensis* and *L. graminifolia* were unsuccessful (table 2).

TABLE 2.—*Summary of hybridization experiments with Lactuca species* [1]

Species involved	Results	Cytological condition of F_1
L. sativa × *L. scariola*	Fertile seed	Regular pairing
L. sativa × *L. chondrillaeflora*	No seed	
L. sativa × *L. perennis*	do	
L. sativa × *L. canadensis*	do	
L. sativa × *L. graminifolia*	do	
L. sativa × *L. cretica*	do	
L. scariola × *L. canadensis*	do	
L. scariola × *L. graminifolia*	do	
L. graminifolia × *L. canadensis*	Fertile seed	Regular pairing

[1] In all of the cases listed, at least 6 flower heads were pollinated. Reciprocal pollinations were made except in the case of *L. sativa* × *L. chondrillaeflora*.

Lactuca perennis.—Of the nine chromosomes of this species, two are markedly shorter than the rest. All of the bivalents are in the form of rings or chains at diakinesis (fig. 2, *D*). Plants of *L. perennis* have not produced seed, yet there is a high percentage of pollen fertility (90 percent). In this case the inability to produce viable seed is evidently not conditioned by chromosomal irregularities. The chromosome complement of this species differs in several respects from that of *L. sativa:* (1) In morphology; (2) in method of pairing, i. e., in *L. perennis* the bivalents are either in rings or chains; and (3) in number of chiasmata per bivalent (*L. perennis*, 1.5; *L. sativa*, 2.0).

FIGURE 2.—Chromosomes from pollen mother cells of *Lactuca* species (× 2,000): *A*, *L. scariola*, diakinesis; *B*, F_1 (Imperial 152 × *L. scariola*), diakinesis, showing perfect pairing of all bivalents; *C*, *L. chondrillaeflora*, metaphase; *D*, *L. perennis*, diakinesis; *E*, *L. tartarica*, diakinesis; *F*, *L. cretica*, diakinesis.

Attempts have been made to cross *L. perennis* with *L. sativa*. Reciprocal matings were made in which two strains of *L. sativa* (Imperial 152 and Imperial D) were used. The results were negative.

Lactuca tartarica.—The chromosome complement of this blue-flowered *Lactuca* is comparable in many respects to that of *L. sativa*, or *L. scariola*. It has nine meiotic chromosomes, of which at least three are considerably longer than the rest (fig. 2, *E*).

MEIOSIS IN THE EIGHT-CHROMOSOME SPECIES

Lactuca cretica.—This large-flowered annual *Lactuca* has eight chromosomes at meiosis; four of the bivalents are of about equal size and somewhat longer than the remainder (fig. 2, *F*). Attempts have been made to cross this species with the nine-chromosome *L. sativa*, and the tetraploid, *L. canadensis*, without success (table 2). The writers have not been able to effect hybridization of *L. cretica* with the other eight-chromosome species, *L. bourgaei*. *L. cretica* flowers early in the spring and dies, whereas *L. bourgaei* comes into flower in late summer or early fall.

Lactuca bourgaei.—This striking perennial, blue-flowered *Lactuca* has eight bivalents of approximately equal length (fig. 3, *A*). Pollen fertility (80 to 85 percent) is not quite as high as in the majority of the species of the genus. This amount of sterility is probably the result of the irregularities found during meiosis. The most common type, the formation of chromatin bridges, is illustrated in figure 3, *B*.

MEIOSIS IN THE 17-CHROMOSOME SPECIES

Lactuca canadensis and *L. graminifolia* [4] have 17 chromosomes at meiosis (fig. 3, *C* and *D*). There is considerable similarity in the chromosome complement of the two species. A study of meiosis in both species indicates that the chromosomes are paired as bivalents. Occasional multivalent associations have been noted, but these are comparatively rare. Pollen fertility in these species ranges from 80 to 95 percent, as shown in table 1. In the F_1 hybrid, pairing is regular (fig. 3, *E*). The percentage of pollen sterility and the production of viable seed indicate no loss of fertility.

The writers have had under observation at least 100 F_2 plants, and all of them appear to be normal. As a result of cytological observations the writers can state that the formation of multivalents is rather rare but that in some cases it is greater than that found in the parent species. In table 3 are summarized the cytogenetic observations of the F_1 and F_2 of the cross *Lactuca canadensis* × *L. graminifolia*.

TABLE 3.—*Cytogenetic observations of the progeny from the cross L. canadensis* × *L. graminifolia*

Progeny	Meiotic behavior	Viable pollen	Seed		Vigor
			Production	Germination	
		Percent			
F_1	Normal	85-90	Abundant	Good	Equal to either parent.
F_2	do [1]	85-90	do	do	Equal to or surpassing both parental species.

[1] In some cases, quite a number of multivalents.

[4] These two species were crossed at the suggestion of J. B. Norton. In the vicinity of Hartsville, S. C., Mr. Norton has observed plants that appear to be natural hybrids of these two species.

FIGURE 3.—Chromosomes from pollen mother cells of *Lactuca* species (× 2,000): *A*, *L. bourgaei*, diakinesis; *B*, *L. bourgaei*, late anaphase, showing bridge configuration; *C*, *L. canadensis*, diakinesis; *D*, *L. graminifolia*, early metaphase; *E*, F_1 (*L. canadensis* × *L. graminifolia*), diakinesis.

DISCUSSION

Results here reported from interspecific hybridization experiments in *Lactuca*, though not extensive, support the following tentative conclusions: (1) Only a single cross (*L. sativa* × *L. scariola*) was obtained within the group having nine chromosomes; (2) no hybrids were secured between species with nine chromosomes and those with eight chromosomes; (3) pollination of the tetraploids (amphidiploids) with pollen from both eight- and nine-chromosome groups gave negative results; (4) hybrids between the two tetraploids can be obtained easily; and (5) in the two cases where interspecific hybrids were obtained (*L. sativa* × *L. scariola* and *L. canadensis* × *L. graminifolia*), the progeny has been fertile and cytologically stable.

According to Lewitsky (*6*), the more primitive species of a group generally have chromosome complements with individuals of equal sizes and as advances are made toward a greater degree of specialization the individuals of a genom become more and more unequal in size. The size differences are presumed to occur as a result of unequal translocation. If the species of *Lactuca* with nine chromosomes are arranged according to this hypothesis, the varieties of *L. sativa* will occupy the most advanced position, with *L. scariola* next and *L. tartarica*, *L. chondrillaeflora*, and *L. perennis* in about that order.

Cooper and Mahony (*2*) found seven bivalents and a ring of four chromosomes in plants of *Lactuca scariola* var. *integrata*. The writers have been unable to confirm this finding, although they have examined collections from a number of stations in the United States and Europe. Their observations indicate that *L. scariola* is a highly variable species, both in morphological characters and in ability to adapt itself to various ecological situations. It therefore seems quite probable that further investigations may reveal strains with translocations.

It has been demonstrated that desirable genes of *Lactuca scariola* can be introduced into *L. sativa*. Plants with a high degree of fertility and cytologically stable are recovered in subsequent generations. This raises the question whether *L. sativa* can be considered as a species distinct from *L. scariola*. The following evidence indicates very definitely that the relationship is extremely close: (1) They cross readily, with the production of fertile seed; (2) F_1 and F_2 individuals are fertile (regular pairing in F_1); (3) parallel morphological variations found in varieties of *L. sativa* can be detected in collections of *L. scariola*, with the possible exception of variations toward the "heading" type.

The possibilities of introducing favorable genes into cultivated lettuce have not been exhausted. There are a number of other species of *Lactuca*, more or less closely related to *L. sativa*, that may be sources of such genes, and efforts should be made to find whether successful crosses can be obtained. In this respect *L. tartarica*, a species with nine chromosomes, may have potentialities; it is a strong, vigorous, rapidly growing perennial. These qualities if introduced into *L. sativa* might be utilized to good advantage.

The work of Babcock, Stebbins, and Jenkins (*1*) indicates that it may prove profitable to initiate hybridization experiments between certain of the eight- and nine-chromosome species that are thought to

be involved in the origin of the amphidiploid American species. Such hybrids should shed some light on the origin of these species and on the puzzling problem of their relationship to each other.

SUMMARY

A technique is described for the preparation of permanent smears of *Lactuca* material.

A brief description of meiosis in the following species of *Lactuca* is given: *L. sativa*, *L. scariola*, *L. chondrillaeflora*, *L. perennis*, *L. tartarica*, *L. cretica*, *L. bourgaei*, *L. graminifolia*, and *L. canadensis*.

Reciprocal matings involving most of the above species have been tried. Fertile F_1 hybrids that have been obtained are *L. sativa* × *L. scariola* and *L. canadensis* × *L. graminifolia*. The F_1 and F_2 individuals are fertile and no cytological irregularities have been found.

The chromosome ideogram is given for all of the diploid species. The greatest degree of morphological differentiation of the chromosomes was found to occur in varieties of *L. sativa*.

With one exception (*Lactuca perennis*), the species under observation were self-fertile and had a high percentage of pollen fertility. In *L. bourgaei*, an eight-chromosome species the pollen fertility was somewhat reduced (80 to 85 percent), apparently as a result of irregularities during meiosis.

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BREED DIFFERENCES IN RESISTANCE TO A DEFICIENCY OF VITAMIN B_1 IN THE FOWL[1]

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INTRODUCTION

The breeds and varieties of domestic fowls differ in size and shape of body, in structure and color of plumage and skin, in variation of the skeleton, and in the modification of structures, such as the comb and spurs, arising from the skin. Apart from colors, the differentiation of the breeds at the time of their establishment was apparently based upon morphological characters. A few differences between breeds in physiological characters, such as broodiness and color of egg, are now known, but it is not impossible that the present comparative freedom of Leghorns from broodiness and the ability of the same breed to lay white-shelled eggs may have resulted largely from artificial selection for those physiological characters as for other distinguishing breed characteristics. This paper reports an association between breed characteristics and an interesting physiological character—resistance to a deficiency of vitamin B_1.

The first indication that White Leghorns require comparatively little vitamin B_1, or through differential storage of it, or by other means, are markedly resistant to polyneuritis, was put forward by Nichita and Iftimesco (*9*).[2] On a diet deficient in vitamin B_1, or lacking it entirely, one of three White Leghorns, 10 months old at the start of the experiment, showed symptoms of polyneuritis after 41 days and died in 49 days. Another was entirely unaffected and was removed from the experiment at 78 days. A third showed no symptoms till the one hundred and seventh day and for 3 weeks thereafter maintained a subacute chronic state of polyneuritis, but quickly recovered when placed on a normal diet. On the other hand, Nichita, Tuschak, and Calcef (*10*) found that on diets deficient in vitamin B_1 six Rhode Island Reds about 10 months old showed symptoms of polyneuritis at from 7 to 19 days. Three of them were given yeast and quickly recovered, but the other three died of acute polyneuritis in 9, 18, and 21 days. In both experiments, controls on the experimental diet plus yeast were entirely normal.

The experiments of Nichita and his coworkers were not set up primarily to test the two breeds as to their requirements of vitamin B_1, and unfortunately the diets used differed considerably. The diet of the Rhode Island Reds included fresh beef, glucose, and starch, whereas that the White Leghorns did not. The latter received their carbohydrates from decorticated rice. Hence, while the experiments within each breed were well controlled, the fact that the diets were not identical makes any comparison of the two breeds with respect to their

[1] Received for publication May 2, 1938. This is No. 8 in the series by the junior author entitled "Genetics of the Fowl."

[2] Italic numbers in parentheses refer to Literature Cited, p. 316.

requirement of vitamin B_1 less accurate than might be desired. Accordingly, experiments were planned by the present writers to determine in chicks of these two breeds their resistance to a deficiency of vitamin B_1. The results are reported in this paper.

MATERIALS AND METHODS

In four separate trials Single Comb White Leghorn chicks were compared with Rhode Island Red chicks of the same age upon a diet deficient in vitamin B_1. Barred Plymouth Rocks were also included in the fourth trial. Environmental conditions were made identical for all chicks in any one experiment by brooding both lots together in the same brooder. To obtain samples of each breed affected as little as possible by differences between individuals and strains, the chicks used in each test were picked at random. Their dams were selected entirely at random. The population from which these chicks came included three strains of Rhode Island Reds and three of White Leghorns. The fowls used to produce chicks for any one test were not the same as those used for the other experiments. Since all the adult breeding stock received the same diet, which was made as complete as possible according to present knowledge of requirements for reproduction in the fowl, any possibility of differential viability in the chicks resulting from differences in the "carry-over" of essential nutrients in the egg was reduced to a minimum.

A diet lacking vitamin B_1 was obtained by the method of Keenan, Kline, Elvehjem, and Hart (*6*) who showed that by autoclaving a mixture of ground grains the vitamin B_1 could be completely destroyed without much loss of vitamin B_2 (riboflavin), or of B_4. The diet used was as follows:

	Pounds per 100
Ground yellow corn	40
Standard wheat middlings	20
Ground buckwheat	15
Wheat-germ meal	2
Soybean oil meal	5
Fish meal	7
Meat scrap	2
Liver meal	3
Alfalfa-leaf meal	3
Ground limestone	2
Salt (NaCl)	.5
High potency cod-liver oil	.5

This diet was used with uniformly good results for all chicks in the regular hatches of the department in 1936. It was autoclaved at 120° C. and 15 pounds pressure for 6 hours. An additional 0.25 percent of high potency cod-liver oil was added after autoclaving. This oil contained 250 units of vitamin D and 3,000 units of vitamin A per gram. The records for the controls proved that the diet not thus treated was entirely satisfactory.

That the data reported are an accurate measure of the requirements for vitamin B_1 and are not complicated by error due to the destruction of other nutrients was shown in a separate experiment with Rhode Island Red chicks. Twenty-five chicks receiving the autoclaved ration died at the mean age of 11 days, and all were dead 14 days after hatching. Of 23 chicks receiving the same ration plus 120 micrograms

of synthetic vitamin B_1 (Betabion, Merck) per 100 g of feed, none had died. Of 25 chicks receiving 240 micrograms of synthetic vitamin B_1 per 100 g of feed 2 died at 4 and 5 days of age. The remainder of the chicks appeared quite healthy at the end of the experiment.

The four experiments were so planned that in each one the dietary deficiency was initiated at a different age and (except in experiment 1) in chicks which, before introduction of the deficient diet or afterwards (experiment 2), had been for different periods upon the normal (unautoclaved) diet, with consequent different storages of vitamin B_1 as temporary defence against the deficiency. Only strong, vigorous chicks were used at the start of each trial. The four tests of the two breeds were as follows:

Experiment 1, initiated February 8, 1936. Deficient diet from hatching.
Experiment 2, initiated January 25, 1936. Deficient diet first 2 days; normal feed on the third day; deficient diet thereafter.
Experiment 3, initiated February 1, 1936. Normal feed to 14 days of age; deficient diet thereafter.
Experiment 4, initiated June 10, 1936. Normal feed to 21 days of age; deficient diet thereafter.

In experiments 3 and 4 all the chicks that died and those alive at the end of experiments were sexed. Since no evidence was found that either sex is more susceptible than the other, data for the two sexes were combined for all analyses.

RESULTS

The findings in the first three experiments (table 1) show conclusively that the White Leghorns are more resistant to a deficiency of vitamin B_1 than are the Rhode Island Reds.

TABLE 1.—*Comparative survival of Single Comb White Leghorn and Rhode Island Red chicks on diets lacking vitamin B_1*

Diet and breed	Chicks	Average body weight at—		Age at death	
		1 day	1 week	Range	Mean
Experiment 1. B_1-free diet from hatching:	*Number*	*Grams*	*Grams*	*Days*	*Days*
White Leghorn	21	35.0	41.1	5–26	12.86
Rhode Island Red	20	36.1	39.0	9–16	10.85
Experiment 2. B_1-free diet from hatching, normal diet on third day only:					
White Leghorn	15	32.0	46.5	15–62	22.13
Rhode Island Red	15	32.3	40.3	13–21	16.93
Experiment 3. Normal diet to 2 weeks, B_1-free diet thereafter:					
White Leghorn	12	36.1	44.1	29–49	34.17
Rhode Island Red	13	35.1	40.8	26–34	29.0

As was to be expected, the mean age at death is lowest for the chicks which never received any normal feed, highest for those on normal feed to 2 weeks of age, and intermediate for those which got normal feed on 1 day only. In each experiment, however, the mean age at death was higher for the White Leghorns than for the Rhode Island Reds. It is noteworthy also that the variation in susceptibility among individuals, as measured by the range in age at death, was much greater in the former than in the latter. In experiments 1, 2, and 3, chicks of the latter breed all died within 8, 9, and 9 days, respectively, whereas

deaths in the White Leghorns in the same trials were spread over 22, 48, and 21 days. The most resistant White Leghorn outlived the last surviving Rhode Island Red by 10, 41, and 15 days in experiments 1, 2, and 3, respectively. An exceptional White Leghorn chick in experiment 2 survived to 62 days of age.

It is commonly believed that animals growing most rapidly are more susceptible to nutritional deficiencies than are those in which growth proceeds more slowly. Weights of these chicks at 1 day (table 1) show the samples of the two breeds to be almost identical in size at the

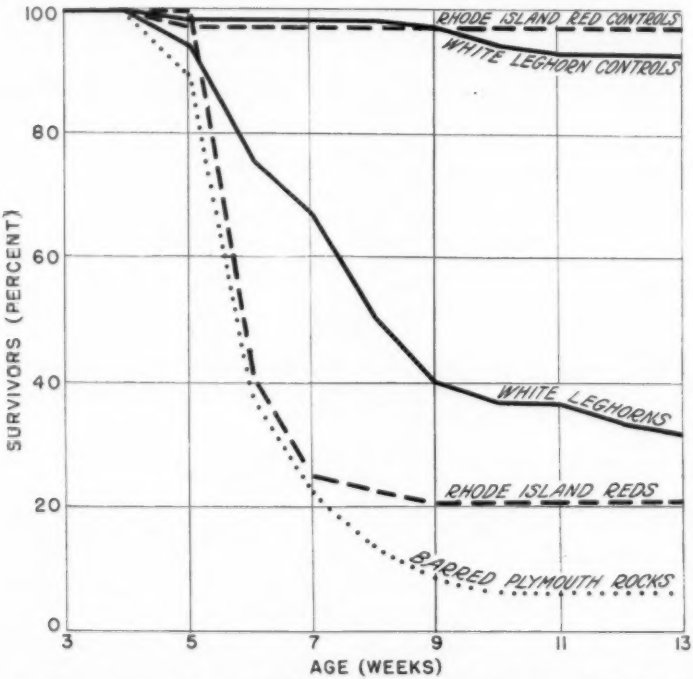

FIGURE 1.—Survival data for 59 White Leghorns, 39 Rhode Island Reds, and 38 Barred Plymouth Rocks placed on vitamin B_1-deficient feed at 3 weeks of age. The controls include 128 White Leghorns and 67 Rhode Island Reds.

beginning of the three tests. The increases in weights of these chicks from hatching to 1 week of age indicate that in all three experiments the White Leghorns grew somewhat more rapidly than the Rhode Island Reds. Ordinarily White Leghorns do not grow more rapidly than Rhode Island Reds in the first week. The differences observed may have resulted from chance or from adverse effects of the vitamin deficiency on the Rhode Island Reds. In any case, the greater resistance of the White Leghorns to a deficiency of vitamin B_1 cannot be ascribed to relatively slow growth in that breed.

Similar results were obtained in experiment 4. Since all the chicks did not die, the results in this test are not strictly comparable with

those in the first three and are therefore presented separately (figs. 1 and 2). Birds in this experiment were kept in battery brooders till 6 weeks old and confined thereafter in a 10-by-12-foot colony house with access to a wire-floored sun porch. Several cases of cannibalism occurred at from 8 to 12 weeks of age, and it is probable that some of the birds still alive after 10 weeks on the vitamin B_1-free diet (at 13 weeks of age, when the experiment was discontinued) were able to survive because of having received an extra supply of the vitamin from the birds eaten. Since these birds went out of the battery brooders and into the colony house at 6 weeks of age, it is possible that after that time some of the birds which survived the longest acquired a supply of the vitamin by coprophagy. It has not yet been proven,

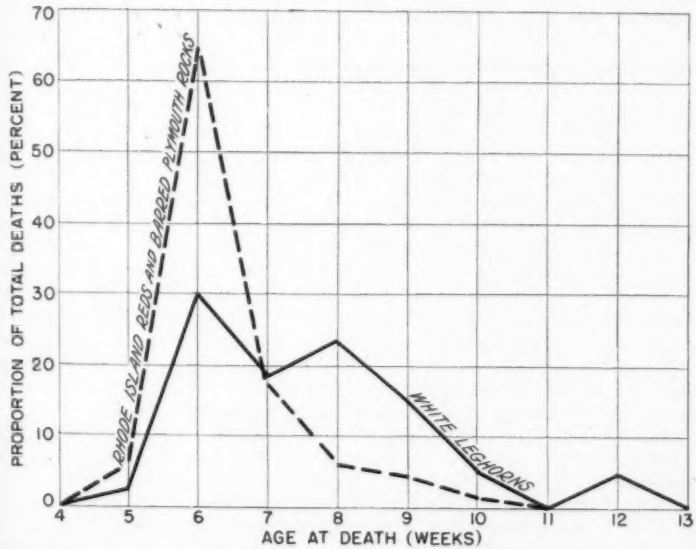

FIGURE 2.—Distribution of ages at death in 39 White Leghorns and 65 Rhode Island Reds and Barred Plymouth Rocks that died from deficiency of vitamin B_1.

however, that in the fowl, as in the rat (*3*) vitamin B^1 is synthesized in the digestive tract and voided with the feces. However, the critical period in this experiment was when the birds were from 5 to 7 weeks of age, at which time the greatest mortality was experienced by all three breeds. Since cannibalism was not serious till the ninth week, it is improbable that error from this source could have affected in any way the breed difference so conclusively demonstrated at from 5 to 8 weeks (fig. 1).

It is quite evident that whatever storage of vitamin B_1 had been accumulated by these chicks during 3 weeks on normal feed it was quickly exhausted (fig. 1). Some mortality occurred in the fourth week, but in the sixth week (third week on the deficient diet) mortality was extremely high in both the Barred Plymouth Rocks and the Rhode Island Reds. Only 39.5 and 41 percent, respectively, of these sur-

vived at the end of the sixth week of the 37 Rhode Island Reds and 34 Barred Plymouth Rocks placed on the deficient diet at 3 weeks of age. In contrast to these, 76.3 percent of the 59 White Leghorns put on the B_1-free diet at 3 weeks were still alive at the end of the sixth week. The percentages of survivors in the Rhode Island Reds, Barred Plymouth Rocks, and White Leghorns at 8 weeks of age were 23.1, 15.8, and 50.8, and at 13 weeks 20.5, 7.9, and 32.2, respectively, of the original numbers in each breed.

The greater resistance of the White Leghorns to a deficiency of this vitamin is demonstrated not only by the lower mortality in that breed but also by the fact that even those that did succumb were able to survive for a longer period than the Rhode Island Reds which died. Analyses of the ages at death up to 13 weeks show that, whereas 64.6 percent of the Plymouth Rocks and Rhode Island Reds that died did so during the sixth week, only 30.8 percent of the White Leghorns that succumbed did so during the sixth week and the rest lived for periods varying up to 7 weeks after the first peak of mortality. As a result the distributions of the ages at death in the two breeds differ markedly (fig. 2). Both curves are skewed; but the curve for the Plymouth Rocks and Rhode Island Reds is leptokurtic, while that for the White Leghorns is platykurtic.

Most of the chicks that died exhibited symptons of vitamin B_1 deficiency including unsteady gait, other signs of neuromuscular incoordination, and, in extreme cases, retraction of the head in characteristic opisthotonos. The birds appeared not to relish the autoclaved feed. This may have resulted from the fact that autoclaving made the mash dark in appearance and (presumably) less palatable, particularly to those chicks which had received normal feed before going on the autoclaved diet. On the other hand, the condition of anorexia may have resulted, in part or entirely, directly from lack of vitamin B_1 as Cowgill (*2*) has shown may happen in other species.

Other evidence of a deficiency of vitamin B_1 was provided by the retardation in growth of the experimental chicks. At 5 weeks of age, just prior to the onset of heavy mortality, the mean weights of the White Leghorns and Rhode Island Reds were respectively 87 and 97 g less than the normal weights for these breeds at 5 weeks, as determined by Card and Kirkpatrick (*1*). Since at 3 weeks of age, when these chicks were placed on the deficient diet, they had exceeded Card and Kirkpatrick's normal weights by 16.5 g in the White Leghorns and 13.7 g in the Rhode Island Reds, the experimental chicks obviously showed at 5 weeks a marked retardation of growth such as is commonly associated with a deficiency of vitamin B_1.

It seems reasonable to conclude, therefore, that the deaths in these chicks resulted primarily from polyneuritis attributable to the deficiency of vitamin B_1.

BREED DIFFERENCES ON A NORMAL DIET

It seemed possible that the differences between breeds with respect to mortality rate might be present even in birds raised under optimum conditions. If this were so, the differences found in experiments 1 to 4 might reflect a general debility of the Rhode Island Reds in comparison with White Leghorns, rather than a special susceptibility to a deficiency of vitamin B_1. To answer this question a comparison was made

of control populations of each breed drawn at random from the several thousand chicks hatched for other purposes from the same strains as those providing the chicks for these experiments.

The mortality curves for these populations show that the strains of White Leghorns and Rhode Island Reds used did not differ in this respect when on normal feed (fig. 1). Up to 3 weeks of age none of either breed died, and at 13 weeks the deaths were 1.5 percent in the Rhode Island Reds and 4.8 percent in the White Leghorns. The diet used for these controls was the same as that of the experimental birds except that it was not autoclaved. It was obviously satisfactory.

It seems safe to conclude that chicks of the two breeds used did not differ in mortality from other causes and that the differences observed in them on the experimental diets can be attributed solely to a difference between the breeds in susceptibility to a deficiency of vitamin B_1.

INDEPENDENCE OF SUSCEPTIBILITY AND BODY SIZE

The demonstration by Nichita et al. (*9, 10*) that adult Rhode Island Reds are more susceptible than adult White Leghorns to a lack of vitamin B_1 indicates a difference between breeds but raises the question whether or not that difference depends upon anything other than the difference in size of those two breeds. Cowgill (*2*) has presented some data indicating that in the larger species the amount of vitamin B_1 required per animal per day is somewhat less in proportion to body weight than in small ones. If this same principle were applicable to intraspecific races differing markedly in size, one would expect a somewhat lower requirement per unit of weight for Rhode Island Reds than for White Leghorns since the former breed exceeds the latter in weight at maturity by an average amount (in females) of around 900 g. This would be the reverse of what has actually been found.

However, the validity of Cowgill's formulas, which are based upon rather hypothetical "maximum normal weights" of the species considered, is open to question. Direct proof that the lower requirement of vitamin B_1 by White Leghorns is independent of their smaller body size at maturity is provided when the experimental animals are of the same size in the breeds compared. It was with this fact in mind that the experiments reported in this paper were carried out with young chicks. Comparisons of growth in White Leghorns and Rhode Island Reds made by Card and Kirkpatrick (*1*) and by Kempster and Parker (*7*) have shown that chicks of the two breeds do not differ in size till after 4 weeks. The present writers' data agree with their findings.

It follows, therefore, that in experiments 1 and 2, where all chicks but one died earlier than 4 weeks of age, and in experiment 3, where the mean age at death in the resistant white Leghorns was not quite 5 weeks, the markedly superior ability of the White Leghorns to withstand a deficiency of vitamin B_1 is a distinct racial characteristic entirely independent of body size. As it happened, the White Leghorns were slightly heavier than the Rhode Island Reds at 1 week of age in these three tests (table 1). In experiment 4, where the deficient diet was not introduced till the chicks were 3 weeks of age, the chicks of the two breeds on hand at 5 weeks of age weighed practically the same, 142.9 g for the Leghorns and 136.4 g for the Rhode Island

Reds. It was in the succeeding 2 weeks that both breeds experienced their heaviest losses (figs. 1 and 2). Obviously the superiority of the White Leghorns in this test was in no way related to body size.

CROSS OF RESISTANT × SUSCEPTIBLE BREEDS

While the primary object of this investigation was to determine whether or not there is a difference between White Leghorns and Rhode Island Reds with respect to their requirement of vitamin B_1, a test was also made to get some idea of the genetic basis for the lower requirement of the White Leghorns. The chicks used were from a cross of Rhode Island Red ♀♀ × White Leghorn ♂. These were tested concurrently with the purebreds in experiment 4 and in exactly the same manner. Mortality in the 53 cross-bred chicks was 73 percent up to 13 weeks of age, when the test was discontinued. The mortality in the same period was 68 percent for purebred White Leghorns and 79.5 percent for purebred Rhode Island Reds. It would appear, therefore, that the crossbred chicks were intermediate between the parent breeds in requirement of vitamin B_1. While further data are necessary to prove the point, this is some indication that the comparatively low requirement of this vitamin by the White Leghorns depend upon multiple factors.

DISCUSSION

These experiments with chicks yielded the same results with respect to breed differences in requirement of vitamin B_1 as did those of Nichita and Iftimesco (*9*) and Nichita et al (*10*) with adult birds. Since the strains of White Leghorns and of Rhode Island Reds used by the Rumanian workers were quite different from those used by the present authors, and since the birds of each breed used by the present writers came from more than one strain, there can be no doubt that the difference observed is really one between breeds and not merely between strains. The low requirement of vitamin B_1 is as truly a genetic breed character as are the dominant white of White Leghorns and the restricted black of the Rhode Island Reds. Whether it is peculiar to White Leghorns or occurs in all Leghorn varieties regardless of color remains to be determined.

The physiological basis for this special attribute of the White Leghorns is as yet quite unknown, and little or no explanation of its persistence as a breed characteristic can be offered. It is obviously different from practically all other distinguishing breed characteristics in that the latter are maintained by artificial selection, whereas it is impossible to see how the poultrymen, either modern or primitive, who helped to establish the White Leghorn breed, could have consciously selected for a lower requirement of vitamin B_1 as they did for morphological characteristics.

The character under consideration is not the only physiological one in which White Leghorns and Rhode Island Reds differ. Hutt (*4*) has shown that these two breeds differ in the rates at which the body temperature rises during the first 9 days after hatching. He (*5*) has also shown that adult White Leghorns are much more resistant to extreme heat than are Rhode Island Reds and Barred Plymouth Rocks. In a discussion of these and other physiological differences

between breeds, the junior author (*5*) has suggested ways in which such differences might have become established, but none of these can yet be considered as anything more than conjecture. The differences are probably between all Leghorns as one class and the so-called heavy breeds as another, rather than merely between the White Leghorns and the Rhode Island Reds.

So far as the writers can ascertain, apart from the work of Nichita and his coworkers, confirmed in this paper, it has not previously been demonstrated that breeds of the domestic fowl vary in their requirement of certain vitamins. Since most recent investigations of the nutritional requirements of fowls are based upon experiments with White Leghorns, there is much doubt about the extent to which findings reported for that breed are applicable to Rhode Island Reds and to other heavy breeds. There has been a tendency in recent years to establish standard requirements of various vitamins for poultry, i. e., so many units of vitamin A, of vitamin D, or of riboflavin per gram of feed. It is obvious that, with respect to vitamin B_1, any such requirement determined from experiments with White Leghorns only would not be accurate for Rhode Island Reds and vice versa.

Assay of vitamin B_1 with pigeons, according to the method of Kinnersley, Peters, and Reader (*8*) is now a standard procedure. These investigators tested pigeons of several different colors, but, so far as the present writers are aware, no experiments have yet been made to determine whether all of the many different breeds of pigeons have the same requirement of this vitamin, or whether some would yield entirely different results in such an assay. It is not impossible that some of the differences in such assays reported from different laboratories have arisen because the pigeon is not yet so well standardized as the commonly used rats of the Wistar Institute strain.

SUMMARY

In each of four experiments the ability of White Leghorn chicks to survive on a diet deficient, or lacking, in vitamin B_1 was greater than that of Rhode Island Reds. When chicks received the deficient diet from hatching, the mean ages at death were 12.9 days for the White Leghorns and 10.8 days for the Rhode Island Reds. For chicks similarly treated but given nondeficient feed on the third day only, the corresponding mean ages were 22.1 and 16.9 days. When deficient feed was supplied after the chicks had been for 2 weeks on the normal diet, the mean ages at death were 34.1 days in the White Leghorns and 29 days in the Rhode Island Reds. Typical symptoms of polyneuritis were observed.

Among chicks on normal feed to 3 weeks of age and the deficient diet thereafter, the percentages surviving to 13 weeks of age were 32 for the White Leghorns, 20.5 for the Rhode Island Reds, and 8 for Barred Plymouth Rocks.

In the White Leghorns deaths were delayed and spread out over a longer period, while in the heavy breeds they occurred earlier and were clustered somewhat narrowly around the mean age at death.

The superior resistance of the White Leghorns to this dietary deficiency is shown to be independent of body size and is not caused by differential mortality from causes other than polyneuritis.

F_1 progeny from a cross of Rhode Island Red ♀♀ × White Leghorn ♂ were intermediate between the parent breeds with respect to resistance to a deficiency of this vitamin.

These findings, along with those of Nichita and his coworkers in Rumania, prove that White Leghorns differ from Rhode Island Reds and Barred Plymouth Rocks in possessing as a hereditary breed characteristic a marked resistance to a deficiency of vitamin B_1.

The significance of this fact in poultry feeding, in experimental work in nutrition, and in the use of birds for quantitative assays of vitamin B_1 is discussed.

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